The Journal of Experimental Biology 207, 3155-3162
Published by The Company of Biologists 2004
doi:10.1242/jeb.01114

Review

Hypoxia tolerance in mammalian heterotherms

K. L. Drew[1,*], M. B. Harris[1], J. C. LaManna[2], M. A. Smith[3], X. W. Zhu[3] and Y. L. Ma[1]

[1]*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775, USA,* [2]*Department of Neurology* and [3]*Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA*

*Author for correspondence (e-mail: ffkld@uaf.edu)

Accepted 26 May 2004

Summary

Heterothermic mammals tolerate severe hypoxia, as well as a variety of central nervous system insults, better than homeothermic mammals. Tolerance to hypoxia may stem from adaptations associated with the ability to survive hibernation and periodic arousal thermogenesis. Here, we review evidence and mechanisms of hypoxia tolerance during hibernation, euthermy and arousal in heterothermic mammals and consider potential mechanisms for regenerative-like processes, such as synaptogenesis, observed within hours of hypoxic stress associated with arousal thermogenesis.

Key words: hibernation, ischemia, JNK/SAPK, inflammation, reoxygenation, hypothermia, antioxidant defense, metabolic suppression.

Introduction

Impaired oxygen delivery is a component of most types of central nervous system (CNS) injury including stroke, global ischemia (cardiac arrest), perinatal hypoxia-ischemia and trauma where blood flow to or within the brain is compromised. Better understanding of mechanisms of hypoxia tolerance may thus lead to improved therapies for a host of CNS injuries. Here, we evaluate tolerance to severe hypoxia in heterothermic[1] mammals. We review evidence and mechanisms of tolerance in the hibernating state, the euthermic state and during the process known as arousal thermogenesis, where heterotherms spontaneously and periodically re-warm every 2–3 weeks throughout the 8-month hibernation season. Finally, we hypothesize how an attenuated stress response during arousal thermogenesis may tip stress-activated protein kinase pathways towards a pro-regenerative outcome and away from neuronal degeneration.

Attenuation of the cytotoxic cascade in hibernating animals

In animals vulnerable to hypoxia, initial disruption of energy balance when oxygen delivery fails to meet demand is the first step in a cascade of events that leads to cell death in metabolically vulnerable cells such as neurons. In the absence of ischemia, glucose is available to maintain ATP levels *via* anaerobic glycolysis. When glucose becomes limiting, however, depletion of high-energy phosphates initiates a cascade of neuropathological events similar to that described in ischemia.

Exhaustion of energy stores rapidly leads to loss of ion homeostasis, causing an influx of Na^+ and Cl^- ions, edema, neuronal depolarization, release of neurotransmitters and opening of voltage-gated ion channels including voltage-gated Ca^{2+} channels. Increases in concentration of glutamate in the extracellular space further stimulate Ca^{2+} influx into neurons. Subsequent generation of reactive oxygen species (ROS), as well as other events, leads to both necrotic and apoptotic processes (Dirnagl et al., 1999). In addition, activation and nuclear translocation of stress-activated protein kinases, nuclear factor κB (NF-κB) and other transcription factors initiate a pro-inflammatory reaction. Intervention at any point of the cytotoxic cascade has the potential to minimize neurological deficit (Fig. 1).

Evidence suggests that adaptations in heterothermic mammals attenuate the cytotoxic cascade at multiple levels (Drew et al., 2001) and differ in many respects from the classic mechanisms of anoxia tolerance in turtles and fish. Heterothermic animals do not have the same glycolytic capacity described for anoxia-tolerant turtles and fish (Lutz and Nilsson, 1997; Perez-Pinzon et al., 1997; Jackson, 2002). Indeed, one aspect of hibernation is a shift from carbohydrate to lipid metabolism (Buck et al., 2002), and hibernation is often associated with a decrease in plasma glucose concentrations (Osborne et al., 1999). Moreover, while heterothermic mammals have an immense capacity to lower body temperature and decrease oxygen demand, especially when hibernating, hypoxia or anoxia does not induce the same degree of

[1]Homeothermy: animal maintains a constant (37°C) body temperature; heterothermy: regulated fluctuation of body temperature between high (37°C) and low (0–37°C); euthermy: state of warm (37°C) body temperature.

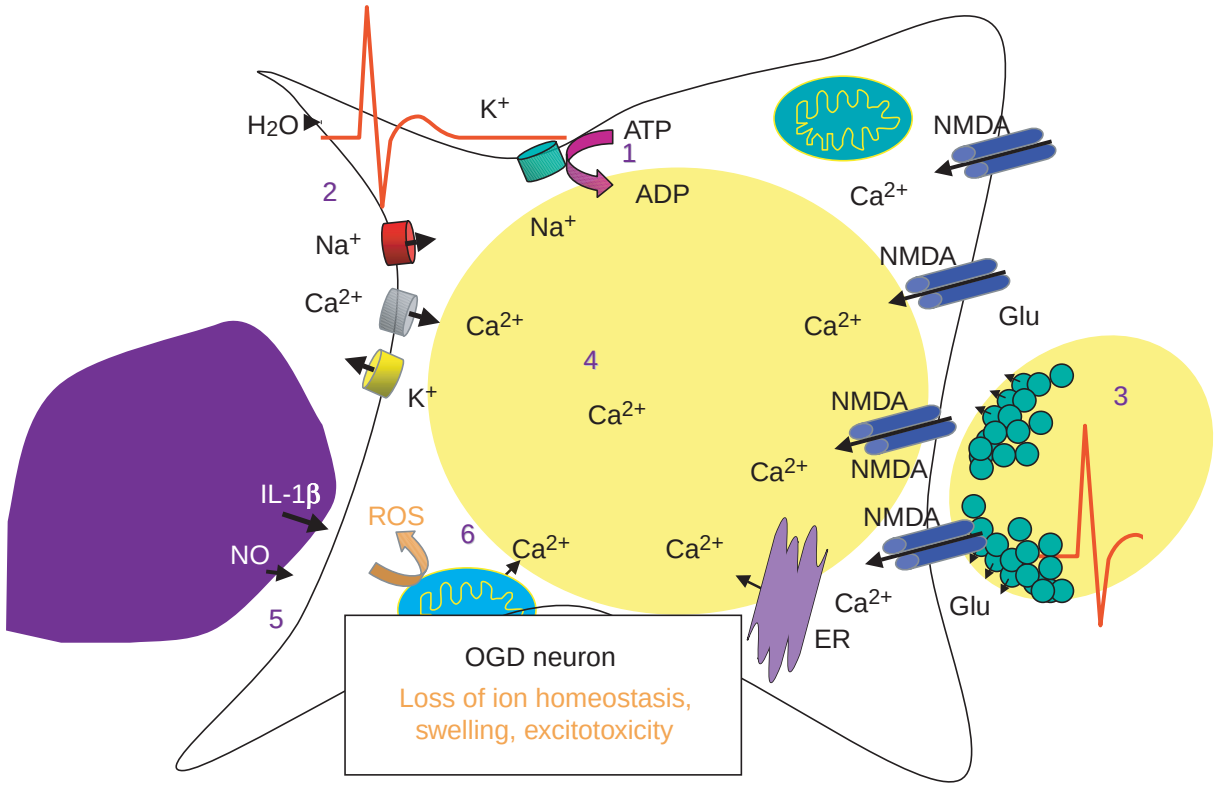

Fig. 1. Anoxia or severe hypoxia typically lead to energy deficit (1), subsequent disruption of ion homeostasis and neuronal depolarization (2, 3). Release of neurotransmitters, including the excitotoxin glutamate, and activation of NMDA and AMPA receptors contribute to the flood of Ca^{2+} from extra- and intracellular stores, which leads to calcium overload (4). Activated microglia release inflammatory cytokines and nitric oxide and contribute to oxidative stress and neuronal cell death (5). Increased reactive oxygen species (ROS) production leads to oxidative modification of cellular components, which contributes to cell death (6). During hibernation, multiple adaptations, in addition to hypothermia, are hypothesized to act in concert to produce pronounced neuroprotection. Decreased demand for oxygen, as well as downregulation of Ca^{2+} channels, maintains energy balance, ion homeostasis and minimizes Ca^{2+} overload. Evidence suggests that immune modulation attenuates inflammatory response, and upregulation of antioxidant defense systems maintains redox balance, thus minimizing the neurodegenerative cascade (Sidky et al., 1972; Spurrier and Dawe, 1973; Drew et al., 1999; Zhou et al., 2001b; Toien et al., 2001). IL-1β, interleukin-1β; NO, nitric oxide; ROS, reactive oxygen species; ER, endoplasmic reticulum; NMDA, *N*-methyl-D-aspartate; Glu, glutamate; OGD, oxygen glucose deprivation.

hypothermia and metabolic suppression in ground squirrels as it does in hypoxia-tolerant turtles and fish (Bullard et al., 1960). Additional adaptations in heterothermic mammals may synergize with hypothermia and metabolic suppression to attenuate the cytotoxic cascade. Because many of these protective mechanisms such as hypothermia, metabolic suppression, immunosuppression/leukocytopenia and increased antioxidant defenses differ between hibernating (torpid) and euthermic animals, we will discuss hypoxia tolerance in hibernating animals separately from hypoxia tolerance in euthermic animals (Fig. 1; reviewed in Drew et al., 2001).

Hypoxia tolerance in the hibernating state: evidence and mechanisms

Reports dating back to the early 1800s describe hypoxia tolerance in the hibernating state in bats, marmots and ground squirrels and show that tolerance displayed during hibernation exceeds tolerance observed during euthermy (Spallanzani et al., 1803; Carlisle, 1805; cited in Biörck et al., 1956; reviewed in Bullard et al., 1960). Biörck et al. (1956) confirmed these initial observations and reported that hibernating hedgehogs (*Erinaceus europaeus*) survive 50–120 min of 100% N_2, in most cases without apparent damage.

Interestingly, many hibernating species in steady-state torpor are *not* hypoxic despite 10-fold or greater decreases in respiratory rates. During torpor, Arctic ground squirrels (*Spermophilus parryii*), as well as other species of ground squirrel, are well oxygenated with normal to above normal arterial oxygen pressures (Frerichs et al., 1994; Y. L. Ma, X. Zhu, P. M. Rivera, O. Toien, B. M. Barnes, J. C. LaManna, M. A. Smith and K. L. Drew, manuscript submitted for publication). By contrast, other heterothermic species, such as golden-mantled ground squirrels (*Spermophilus lateralis*) and hedgehogs (*E. europaeus*), may become hypoxic during torpor owing to long periods of apnea. These species often breathe intermittently during hibernation, waiting up to 30 min or longer between breaths. In hedgehogs, arterial oxygen partial pressure (Pa_{O_2}), sampled from chronic aorta cannula, is higher in torpor than in the active state (120 *vs* 105 mmHg; 160 *vs* 14.0 kPa) but

falls to 10 mmHg (1.3 kPa) at the end of the apneic period lasting 50–70 min (Tahti and Soivio, 1975). It is unclear if tissue becomes hypoxic during periods of apnea since neither tissue lactate nor tissue oxygen tension has been reported. Hemoglobin O_2 affinity is typically higher in hibernating species and even higher at cold temperatures. Thus, tissue hypoxia may not be as high as indicated by the low Pa_{O_2}. In hibernating golden-mantled ground squirrel, the P_{50} at 7°C and a pH of 7.46 was found to be 5.8 mmHg (0.77 kPa; Maginniss and Milsom, 1994).

Neuroprotection against hypoxia during hibernation is thought to result from synergy between multiple adaptations, including extreme hypothermia (beyond what is tolerated by homeotherms), increased antioxidant defense, metabolic suppression, immune modulation and decreased ion channel activity (Fig. 1; Drew et al., 2001). Interestingly, one paradigm of ischemic preconditioning, found to attenuate volume of infarction by ~60%, induced changes in gene expression reminiscent of adaptations observed in hibernating animals (Stenzel-Poore et al., 2003). Using microarray analysis, preconditioning was found to induce changes in gene expression consistent with suppression of metabolic pathways and immune responses and reduction of ion channel activity (Stenzel-Poore et al., 2003), all of which are characteristic of hibernation and thought to contribute to neuroprotection in the hibernating state (Drew et al., 2001). Stenzel-Poore et al. (2003) suggest that both hibernation and the protein-synthesis-dependent preconditioning observed in their study are associated with an evolutionarily conserved reprogramming of the cytotoxic cellular response. Of the numerous adaptations exhibited by hibernating animals, metabolic suppression may be the most novel and least well mimicked by current pharmacotherapies.

Mechanisms of metabolic suppression

During hibernation in Arctic ground squirrels, oxygen consumption decreases from ~1 to 0.01 ml O_2 g^{-1} h^{-1} (Buck and Barnes, 2000; Toien et al., 2001), electroencephalogram (EEG) is isoelectric (Frerichs et al., 1994) and heart and respiratory rates decrease by ⩾10-fold (Y. L. Ma, X. Zhu, P. M. Rivera, O. Toien, B. M. Barnes, J. C. LaManna, M. A. Smith and K. L. Drew, manuscript submitted for publication). It would be expected that this suppression in oxygen demand would enhance tolerance to low oxygen supply during hibernation. Indeed, metabolic depression appears to preserve arterial oxygen tension during steady-state torpor when respiratory rates can fall to less than one breath per minute. The contribution of additional metabolic depression during apnea in hibernating animals is unclear.

Mechanisms of metabolic suppression in hibernation are poorly understood. During entrance into or arousal from hibernation, changes in oxygen consumption, heart rate, respiratory rate and cerebral blood flow all precede changes in core body temperature (Lyman, 1982; Toien et al., 2001; Osborne and Hashimoto, 2003). These results argue for active regulation of metabolism beyond that of temperature effects and support the model that parasympathetic tone coordinates entrance into and maintenance of torpor while an increase in sympathetic tone initiates arousal (Twente and Twente, 1978; Harris and Milsom, 1995; Milsom et al., 1999). Further evidence for regulated metabolic suppression that goes beyond temperature effects comes from observations in the Arctic ground squirrels, where body temperature and metabolic rate dissociate during steady-state torpor over a range of ambient temperatures (Buck and Barnes, 2000). While metabolic suppression during steady-state torpor cannot be explained entirely by temperature effects in Arctic ground squirrels, or in other small heterotherms (Geiser, 1988), this may not be the case in all heterothermic species. Zimmer and Milsom (2001) reported that in golden-mantled ground squirrels oxygen consumption parallels core body temperature, suggesting that, in this species, steady-state metabolism is not lower than what is achieved through temperature-dependent suppression of biochemical processes (Q_{10} effects).

A mechanism that may be central to metabolic suppression during entrance into and maintenance of torpor involves a change in thermoregulatory set point (Florant and Heller, 1977). The mammalian thermostat is located in the preoptic anterior hypothalamus (POAH), and cooling this area below the thermoregulatory set point evokes thermogenesis, indicated by an increase in oxygen consumption. The thermoregulatory set point (i.e. the temperature of the POAH that evokes thermogenesis) decreases as animals enter hibernation, and this decrease precedes the decrease in body temperature. Turning down the thermostat abruptly decreases oxygen consumption and invokes coordinated cooling of core body temperature *via* shunting of core blood to the periphery to facilitate heat loss (Heller et al., 1977; Florant and Heller, 1977). The subsequent drop in body temperature then facilitates metabolic suppression through thermodynamic effects on metabolic processes (Geiser, 1988). This ‘black box’ thermostat thus appears to play a major role in metabolic suppression in hibernation and metabolic response to hypoxia in the euthermic state (discussed below). Unveiling mechanisms of the thermostat, as well as the means that hibernating species use to tolerate such low body temperatures, may lead to therapeutic strategies when oxygen delivery is limited. Stimulation of the cerebellar fastigial nucleus protects against cerebral ischemia in rats (Reis et al., 1997), and this effect may involve suppression of cerebral glucose metabolism. Involvement of this pathway in hibernation has not been studied.

Other potential mechanisms contributing to metabolic suppression in hibernation at the cellular level are ion channel arrest, increase in inhibitory neurotransmission and suppression of substrate oxidation. Evidence of ion channel arrest in hibernation comes from studies of Ca^{2+} uptake in brain and cardiac tissues (Gentile et al., 1996; Wang et al., 2002). Surprisingly, extracellular levels of the inhibitory neurotransmitter GABA *decrease* in striatum during hibernation (Osborne et al., 1999), and extracellular glutamate remains unchanged during steady-state torpor compared with euthermic animals (Zhou et al., 2001a). Finally, studies in Arctic ground squirrel show evidence of tissue-specific

depression of substrate oxidation during hibernation. At an assay temperature of 37°C, state 3 and state 4 respiration decrease in liver mitochondria but not skeletal muscle mitochondria isolated from hibernating ground squirrels (Barger et al., 2003). A decrease in substrate oxidation would decrease oxygen consumption but could be due to a decrease in demand for ATP as well as direct inhibition of the biochemical reactions necessary for substrate oxidation. Barger et al. (2003) found no evidence for a decrease in futile proton leak in hibernating mitochondria.

Importantly, hypoxia, anoxia or other forms of physiological stress are not sufficient to induce hibernation in ground squirrels (Bullard et al., 1960; K. L. Drew, K. Cozad, Y. Ma, P. M. Rivera and H. Zhao, unpublished). This contrasts with hamsters, where, after short-daylight-induced gonadal regression, food and/or water deprivation is sufficient to induce torpor (Lyman and Chatfield, 1955). While it is unclear what signaling events induce hibernation in ground squirrels and other obligatory hibernators, they are linked to circannual rhythm and the reproductive cycle. Gonadal regression and genesis precede hibernation in the autumn and emergence from hibernation in the spring. Non-circadian functions of the suprachiasmatic nucleus may coordinate circannual rhythm (Dark et al., 1990), as well as the timing of interbout arousal episodes that interrupt prolonged torpor throughout the hibernation season (Ruby et al., 2002). While decreased oxygen demand may not explain all of the 28-fold increase in survival time under 100% N_2 in hibernating hedgehogs (Biörck et al., 1956), 10-fold decreases in oxygen consumption certainly have the potential to enhance survival under conditions of limited oxygen and nutrient delivery to vital organs, suggesting that mechanisms of metabolic suppression with potential application in humans warrant further study.

Hypoxia tolerance during euthermy: evidence and mechanisms

Heterothermic species in the euthermic state also survive hypoxia better than non-hibernating species, albeit tolerance is not as dramatic as is seen during hibernation. Hiestand et al. (1950) were the first to systematically compare differences in hypoxia tolerance between hibernating and non-hibernating species in the euthermic state. These authors found that hibernating species – hamster, ground squirrel and bat – survived 106 mmHg (14.1 kPa) atmospheric pressure (P_{O_2}= 21 mmHg or 2.8 kPa) for 8, 18 and 60 min, respectively, whereas rat and guinea pig, the longest survivors of the non-hibernating species, survived for only 1–3 min. Moreover, the hibernating species showed fewer signs of respiratory distress and died slowly and without convulsions. Bats that had been revived and exposed to subsequent hypoxia showed no evidence of acquired hypoxia tolerance (Hiestand et al., 1956).

Do physiological adaptations necessary for successful hibernation, such as the ability to restrict blood flow to vital organs during rewarming and to vasodilate to facilitate body cooling during entrance into hibernation, contribute to hypoxia tolerance? Hypoxia is known to decrease metabolism in many vertebrates through cooling of core body temperature (Barros et al., 2001). Cooling is achieved *via* preference for cooler environments, body posture and a decrease in POAH thermoregulatory set point and subsequent peripheral vasodilation similar to what occurs during entrance into hibernation (Tattersall and Milsom, 2003). Evidence suggests that the hypoxic metabolic response is of greater magnitude in heterothermic species, presumably because the regulatory mechanisms are similar to those used during entrance into hibernation (Bullard et al., 1960; Burlington et al., 1969; Barros et al., 2001). Hypothermia, induced under controlled conditions, improves neurological outcome after hypoxia and ischemia in animal models as well as in humans following cardiac arrest (Busto et al., 1987; Globus et al., 1995; Hypothermia after Cardiac Arrest Study Group, 2002), although the multifactorial mechanisms of protection are still poorly understood (Holzer and Sterz, 2003). Magnitude of cooling may enhance protection until limit of cold tolerance is reached (Huh et al., 2000). Thus, enhanced tolerance to decreased core body temperature due to adaptations at the cellular level may play as great a role in tolerance to hypoxia as the hypoxic metabolic response, because the latter requires low core body temperature to be effective. Finally, coordinated cooling cannot fully explain hypoxia tolerance in euthermic heterotherms. Bullard et al. (1960) found that at all ambient temperatures, including those at which no body cooling was possible, euthermic heterotherms outlived non-hibernating species and concluded that temperature was not the only factor involved in hypoxia tolerance.

Other factors that could contribute to hypoxia tolerance in euthermic heterotherms include higher levels of ketone bodies (D'Alecy et al., 1990), circannual suppression of immune response (Sidky et al., 1972), antioxidant defense (Buzadzic et al., 1997), seasonal changes in metabolism (Boyer et al., 1997) and differences in intrinsic tissue properties. Frerichs and Hallenbeck (1998) provide the only evidence for differences in intrinsic tissue properties: at 36°C, protection from oxygen glucose deprivation in hippocampal slices from euthermic, 13-lined ground squirrels was better than rat (albeit not as good as in slices from hibernating ground squirrels). Importantly, differences between groups (hibernating ground squirrel, euthermic ground squirrel and rat) were enhanced at colder temperatures. Further studies are warranted to confirm intrinsic tissue differences and to address mechanisms of hypoxia tolerance at the tissue level.

One cellular mechanism could involve a preconditioning-like phenomenon. Curiously, cellular stress evidenced by elevated brain tissue levels of iNOS (inducible nitric oxide synthase) and HIF-1α (hypoxia indicible factor 1α) and activation of ERK (extracellular-signal-regulated kinase) and JNK/SAPK (c-Jun N-terminal kinase/stress-activated protein kinase) (Zhu et al., 2004) are consistent with a state of preconditioning in euthermic Arctic ground squirrels. Resting Pa_{O_2} values reported for euthermic mammalian heterotherms are frequently below 80 mmHg (11 kPa; Burlington et al., 1969; Frerichs et al., 1995), and evidence of mild, uncompensated, chronic hypoxia,

indicated by low $P_{a_{O_2}}$, high $P_{a_{CO_2}}$, decreased pH and elevated levels of HIF-1α, is consistently observed in euthermic Arctic ground squirrels (Y. L. Ma, X. Zhu, P. M. Rivera, O. Toien, B. M. Barnes, J. C. LaManna, M. A. Smith and K. L. Drew, manuscript submitted for publication). Although low $P_{a_{O_2}}$ may not translate directly to tissue hypoxia because of increased hemoglobin oxygen affinity observed in other species of heterotherms (discussed above; Maginnis and Milsom, 1994), elevated $P_{a_{CO_2}}$, decreased pH and associated upregulation or activation of stress signaling pathways suggest that euthermic Arctic ground squirrels experience mild, chronic stress. The functional significance and cause-and-effect relationship between low $P_{a_{O_2}}$ and cellular stress in euthermic ground squirrels remain to be determined; however, it is tempting to speculate that mild, chronic hypoxia and associated cellular stress precondition these animals to tolerate more severe hypoxia.

Hypoxia tolerance as an adaptation to heterothermy *per se*

A question, germane to the current discussion, is whether tissues from hibernators are truly hypoxia tolerant when compared with those of other, non-hibernating mammals. Many species of non-hibernating mammals, such as the mole rat, are extremely tolerant of hypoxia (Wildmer et al., 1997; Weibel, 1999). Hypoxia-tolerating characteristics have been attributed to adaptations to chronic hypoxia associated with the burrow-dwelling lifestyles of many fossorial mammals (Boggs et al., 1984; Tenney and Boggs, 1986; Boggs and Birchard, 1989; Mortola, 1991; Frappell and Mortola, 1994). Many heterothermic mammals that exhibit tolerance to hypoxia are also fossorial. Thus, it is not clear whether it is the capacity for heterothermy or the chronic hypoxia of a fossorial life that results in hypoxia tolerance. A number of semi-fossorial, as well as non-fossorial, heterotherms exhibit tolerance to hypoxia comparable with fossorial species (Hiestand et al., 1950; Biörck et al., 1956; Burlington et al., 1971; Davies and Schadt, 1989; Mortola, 1991; Walsh et al., 1996; Boggs et al., 1999; Frappell et al., 2002). Such tolerance does not necessarily depend on exposure to chronic hypoxia, as it is also expressed in adults not exposed to hypoxia during development (Mortola, 1991). A parsimonious conclusion is that the chronic hypoxia of either fossorial life or heterothermy could result in adaptations to hypoxia tolerance in a given species. Regardless of origin, adaptations that facilitate hypoxia tolerance are present in heterothermic species and probably play significant roles in the adaptive physiology of these animals.

Hypoxia tolerance during arousal from hibernation: evidence and mechanisms

As discussed above, hibernation *per se* is not a hypoxic state where, due to decreases in O_2 utilization, $P_{a_{O_2}}$ can exceed normoxic values. However, as Arctic ground squirrels work to re-warm, $P_{a_{O_2}}$ falls to a minimum of 7 mmHg (0.9 kPa) for as long as 60 min at the same time as oxygen consumption peaks. $P_{a_{O_2}}$ then remains below 35 mmHg (4.7 kPa) for as long as 4 h (Y. L. Ma, X. Zhu, P. M. Rivera, O. Toien, B. M. Barnes, J. C. LaManna, M. A. Smith and K. L. Drew, manuscript submitted for publication). Despite this period of severe systemic hypoxia, ground squirrels do not show signs of CA1 or cortical neuronal pathology or oxidative stress. Core body temperature remains less than 20°C during the most severely low levels of $P_{a_{O_2}}$; however, brain temperature, brain $P_{a_{O_2}}$ or brain tissue oxygen tension at this time has not been determined (Y. L. Ma, X. Zhu, P. M. Rivera, O. Toien, B. M. Barnes, J. C. LaManna, M. A. Smith and K. L. Drew, manuscript submitted for publication). Decreased brain temperature may play a role in protection, since even minor (3°C) decreases in brain temperature, as discussed above, protect against pathological effects of ischemia in rodents as well as humans (Busto et al., 1987; Globus et al., 1995; Hypothermia after Cardiac Arrest Study Group, 2002). Selective shunting of blood to the brain and other vital organs is another likely mechanism of cerebral protection during re-warming. The blood flow in the brain reaches a maximum at the same time as the peak in oxygen consumption (Osborne and Hashimoto, 2003), and a diving-like response in blood flow during re-warming has been described (Adolph and Richmond, 1955). Brain hypoxia during arousal in hibernating bats is indicated by increases in brain tissue lactate concentrations, although brain temperature at the time of hypoxia is not known (Lee et al., 2002).

While potential mechanisms of hypoxia tolerance during torpor are numerous and obvious, many of the neuroprotective aspects of hibernation begin to subside during arousal thermogenesis. For example, while metabolic suppression is pronounced during torpor, oxygen consumption peaks as animals work to re-warm, precisely at the time of minimum $P_{a_{O_2}}$ (Ma et al., 2004). Nonetheless, studies in hamsters (*Mesocricetus auratus*) indicate that energy charge in brain is maintained during arousal (Lust et al., 1989). Production of ROS exceeding antioxidant capacity during hypoxia and re-oxygenation damages cellular components directly *via* oxidative modification as well as indirectly *via* activation of inflammatory and pro-death signaling pathways. A generalized adaptation of hypoxia-tolerant animals may be increased antioxidant defense mechanisms to protect cells from ROS during re-oxygenation (Hermes-Lima and Zenteno-Savin, 2002). Ascorbate, one of the most important low-molecular-mass antioxidants in plasma, increases 4-fold in plasma and doubles in cerebral spinal fluid (CSF) during hibernation (Drew et al., 1999; Toien et al., 2001). While ROS generation is expected to be low during hibernation, in part due to suppressed flux of oxygen through the electron transport chain, generation of ROS is assumed to increase during arousal thermogenesis in parallel with pronounced increases in oxidative metabolism. In spite of the surge in metabolism, concomitant with a decline in $P_{a_{O_2}}$ during arousal thermogenesis, no evidence of oxidative modification in brain has been observed following arousal from hibernation (Ma et al., 2004). Evidence suggests that redistribution of ascorbate from plasma to metabolically active tissues during arousal protects tissues from oxidative modification (Toien et al., 2001). Indeed, plasma ascorbate concentrations decline in parallel with peak oxygen consumption

(Toien et al., 2001), and brain ascorbate concentrations increase significantly towards the end of arousal (Ma et al., 2004).

Hypoxia-reoxygenation promotes neuronal injury, in part *via* toxic inflammatory mediators produced by activated microglial cells and infiltrating leukocytes. SAPKs, a family of serine/threonine kinases including JNKs and p38, are part of a phosphorelay system that regulates cellular activities (Johnson and Lapadat, 2002). Activation of JNK and p38 by environmental stressors such as hypoxia often leads to cell death *via* inflammation and apoptosis and comprises part of the signal transduction cascade involved in neurodegenerative hypoxia (Kunz et al., 2001; Fig. 2).

Analysis of SAPK activation in the brain following arousal from hibernation is consistent with activation of an attenuated stress response. Recent results show that, while JNK is activated during arousal, p38 is not activated following arousal in bats or Arctic ground squirrels (Lee et al., 2002; Zhu et al., 2004). Furthermore, iNOS, known to be induced downstream of p38 activation (Park et al., 2002), is not induced by arousal in Arctic ground squirrels (Zhu et al., 2004). Interestingly, circulating leukocytes rapidly return to euthermic values, and the acute-phase response to bacterial lipopolysaccharide (LPS) is fully restored (Toien et al., 2001; Prendergast et al., 2002). Nonetheless, failure to activate p38 or induce iNOS argues for sufficient modulation of the immune response during arousal to prevent a fully developed inflammatory stress response.

Finally, oxygen delivery to tissues may be enhanced during arousal, as hemoglobin with high oxygen affinity in the hibernating state transitions to hemoglobin with lower oxygen affinity in the euthermic state (Maginniss and Milsom, 1994). The combined effects of hypothermia, enhanced antioxidant defense mechanisms, attenuation of the inflammatory response, as well as enhanced oxygen delivery, thus have the potential to provide significant protection against hypoxia during arousal.

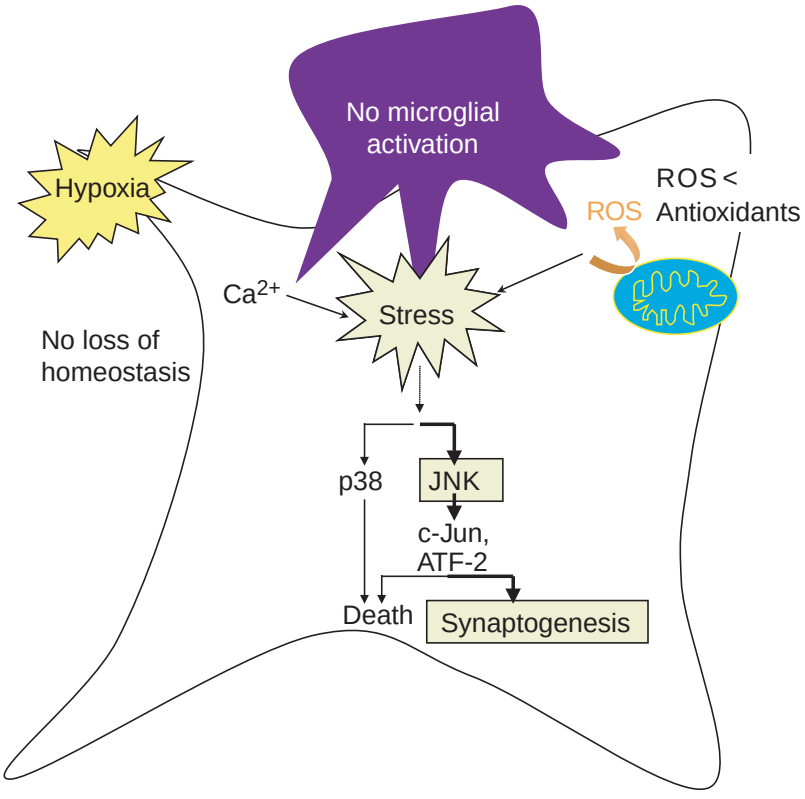

Fig. 2. Hypoxia activates SAPK pathways, leading to neuronal cell death. During arousal thermogenesis, attenuation of SAPK activation due to hypothermia, immune modulation and enhanced antioxidant defenses, such as increased brain ascorbate concentrations, may tip downstream consequences towards a pro-regenerative and/or synaptogenic outcome. JNK, c-Jun *N*-terminal kinase; ATF-2, activating transcription factor 2; ROS, reactive oxygen species.

Regeneration or protection from degeneration? Evidence for synaptogenesis following arousal thermogenesis

Neuronal cell fate is determined by the balance between regenerative and degenerative processes. In addition to attenuation of degeneration reviewed above, adaptation to hypoxia and cellular stress associated with arousal thermogenesis may likewise involve regeneration. Both synaptogenesis (Popov et al., 1992; Popov and Bocharova, 1992; Arendt et al., 2003) and enhanced cognitive function have been observed following arousal (McNamara and Riedesel, 1973; Mihailovic et al., 1968). Elucidation of mechanisms that regulate balance between negative and positive outcome for neurons would provide targets for therapeutic intervention following hypoxia and other forms of CNS trauma.

JNK/SAPK is a potential signaling crossroads between degeneration and regeneration that may be tipped towards a regenerative outcome under physiological conditions of arousal thermogenesis. JNK/SAPK is one of the major molecules activated by deleterious stimuli such as UV irradiation, hypoxia, free radicals and cytokines. Although, in many cases, the activation of JNK/SAPK leads to cell death, such as is seen in hypoxia-induced apoptosis in hepatocytes and developing brain neurons (Crenesse et al., 2000; Chihab et al., 1998; Kunz et al., 2001), the activation of JNK/SAPK has also been shown to mediate hypoxia-induced expression of basic fibroblast growth factor (bFGF) and hypoxia-induced proliferative responses of fibroblasts (Das et al., 2001; Le and Corry, 1999). More recently, JNK activity has been shown to be essential for late-stage neuritogenesis in N1 cell cultures and suggested to be involved in late stages of functional differentiation such as synaptic connection formation (Xiao and Liu, 2003). JNK activation, in the absence of neuronal pathology during arousal (Ma et al., 2004; X. Zhu, M. A. Smith, G. Perry, Y. Wang, P. M. Rivera, A. P. Ross, H. W. Zhao, J. C. LaManna and K. L. Drew, manuscript submitted for publication), and synaptogenesis shortly after arousal (Popov et al., 1992) argue that activation of JNK/SAPK during arousal may reflect an effort to mobilize regenerative rather than apoptotic processes.

In summary, heterothermic mammals possess a repertoire of neuroprotective adaptations that are hypothesized to contribute to hypoxia tolerance. Tolerance is most pronounced in the hibernating state, although hibernating animals are not hypoxic due to ⩾10-fold decreases in oxygen demand. Like hibernating animals, euthermic heterotherms tolerate hypoxia better than homeotherms. This tolerance is hypothesized to stem from adaptations necessary for successful hibernation. Curiously, euthermic Arctic ground squirrels appear to be mildly, but chronically, hypoxic at normal atmospheric pressures and oxygen tensions. The ability to tolerate hypoxia may be necessary for successful arousal thermogenesis, where P_{aO_2} decreases in parallel with increased oxygen consumption. It is hypothesized that, during arousal, selective activation of JNK/SAPK *via* attenuation of hypoxia-induced stress tips the outcome of activation of SAPK towards regeneration.

Authors acknowledge secretarial assistance from Leah Swasey. This work was supported by NIH-NS41069 (NINDS, NIMH, NCRR and NCMHD) to K.L.D. and M.A.S. and NIH-NS38632 (NINDS) to J.C.L.

References

Adolph, E. F. and Richmond, J. (1955). Rewarming from natural hibernation and from artificial cooling. *J. Appl. Physiol.* **8**, 48-58.

Arendt, T., Stieler, J., Strijkstra, A. M., Hut, R. A., Rudiger, J., Van der Zee, E. A., Harkany, T., Holzer, M. and Hartig, W. (2003). Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J. Neurosci.* **23**, 6972-6981.

Barger, J. L., Brand, M. D., Barnes, B. M. and Boyer, B. B. (2003). Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **284**, R1306-1313.

Barros, R. C., Zimmer, M. E., Branco, L. G. and Milsom, W. K. (2001). Hypoxic metabolic response of the golden-mantled ground squirrel. *J. Appl. Physiol.* **91**, 603-612.

Biörck, G., Johansson, B. and Schmid, H. (1956). Reactions of hedgehogs, hibernating and non-hibernating, to the inhalation of oxygen, carbon dioxide and nitrogen. *Acta Physiol. Scand.* **37**, 71-83.

Boggs, D. F. and Birchard, G. F. (1989). Cardiorespiratory responses of the woodchuck and porcupine to CO_2 and hypoxia. *J. Comp. Physiol. B* **159**, 641-648.

Boggs, D. F., Kilgore, D. L. and Birchard, G. F. (1984). Respiratory physiology of burrowing mammals and birds. *Comp. Biochem. Physiol. A* **77**, 1-7.

Boggs, D. F., Maginniss, L. A. and Kilgore, D. L., Jr (1999). In vivo blood oxygen binding in hypoxic lesser spear-nosed bats: relationship to control of breathing. *Respir. Physiol.* **118**, 193-202.

Boyer, B. B., Ormseth, O. A., Buck, L., Nicolson, M., Pelleymounter, M. A. and Barnes, B. M. (1997). Leptin prevents post hibernation weight gain but does not reduce energy expenditure in arctic ground squirrels. *Comp. Biochem. Physiol. C* **118**, 405-412.

Buck, C. L. and Barnes, B. M. (2000). Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **279**, R255-R262.

Buck, M. J., Squire, T. L. and Andrews, M. T. (2002). Coordinate expression of the PDK4 gene: a means of regulating fuel selection in a hibernating mammal. *Physiol. Genomics* **8**, 5-13.

Bullard, R. W., David, G. and Nichols, C. T. (1960). The mechanisms of hypoxic tolerance in hibernating and non-hibernating mammals. In *Mammalian Hibernation. Bulletin of the Museum of Comparative Zoology at Harvard College*, vol. 24 (ed. C. P. Lyman and A. R. Dawe), pp. 321-335. Cambridge, MA: Museum of Comparative Zoology at Harvard College.

Burlington, R. F., Maher, J. T. and Sidel, C. M. (1969). Effect of hypoxia on blood gases, acid–base balance and in vitro myocardial function in a hibernator and a nonhibernator. *Fed. Proc.* **28**, 1042-1046.

Burlington, R. F., Vogel, J. A., Burton, T. M. and Salkovitz, I. A. (1971). Cardiac output and regional blood flow in hypoxic woodchucks. *Am. J. Physiol.* **220**, 1565-1568.

Busto, R., Dietrich, W. D., Globus, M. Y., Valdes, I., Scheinberg, P. and Ginsberg, M. D. (1987). Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J. Cereb. Blood Flow Metab.* **7**, 729-738.

Buzadzic, B., Blagojevic, D., Korac, B., Saicic, Z. S., Spasic, M. B. and Petrovic, V. M. (1997). Seasonal variation in the antioxidant defense system of the brain of the ground squirrel (*Citellus citellus*) and response to low temperature compared with rat. *Comp. Biochem. Physiol. C* **117**, 141-149.

Chihab, R., Ferry, C., Koziel, V., Monin, P. and Daval, J. L. (1998). Sequential activation of activator protein-1-related transcription factors and JNK protein kinases may contribute to apoptotic death induced by transient hypoxia in developing brain neurons. *Brain Res. Mol. Brain Res.* **63**, 105-120.

Crenesse, D., Gugenheim, J., Hornoy, J., Tornieri, K., Laurens, M., Cambien, B., Lenegrate, G., Cursio, R., De Souza, G., Auberger, P. et al. (2000). Protein kinase activation by warm and cold hypoxia-reoxygenation in primary-cultured rat hepatocytes-JNK(1)/SAPK(1) involvement in apoptosis. *Hepatology* **32**, 1029-1036.

D'Alecy, L. G., Lundy, E. F., Kluger, M. J., Harker, C. T., LeMay, D. R. and Shlafer, M. (1990). Beta-hydroxybutyrate and response to hypoxia in the ground squirrel, *Spermophilus tridecimlineatus*. *Comp. Biochem. Physiol.* **96B**, 189-193.

Dark, J., Kilduff, T. S., Heller, H. C., Licht, P. and Zucker, I. (1990). Suprachiasmatic nuclei influence hibernation rhythms of golden-mantled ground squirrels. *Brain Res.* **509**, 111-118.

Das, M., Bouchey, D. M., Moore, M. J., Hopkins, D. C., Nemenoff, R. A. and Stenmark, K. R. (2001). Hypoxia-induced proliferative response of vascular adventitial fibroblasts is dependent on G protein-mediated activation of mitogen-activated protein kinases. *J. Biol. Chem.* **276**, 15631-15640.

Davies, D. G. and Schadt, J. C. (1989). Ventilatory responses of the ground squirrel, *Spermophilus tridecemlineatus*, to various levels of hypoxia. *Comp Biochem. Physiol.* **92A**, 255-257.

Dirnagl, U., Iadecola, C. and Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* **22**, 391-397.

Drew, K. L., Osborne, P. G., Frerichs, K. U., Hu, Y., Koren, R. E., Hallenbeck, J. M. and Rice, M. E. (1999). Ascorbate and glutathione regulation in hibernating ground squirrels. *Brain Res.* **851**, 1-8.

Drew, K. L., Rice, M. E., Kuhn, T. B. and Smith, M. A. (2001). Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. *Free Radic. Biol. Med.* **31**, 563-573.

Florant, G. L. and Heller, H. C. (1977). CNS regulation of body temperature in euthermic and hibernating marmots (Marmota flaviventris). *Am. J. Physiol.* **232**, R203-R208.

Frappell, P. B., Baudinette, R. V., MacFarlane, P. M., Wiggins, P. R. and Shimmin, G. (2002). Ventilation and metabolism in a large semifossorial marsupial: the effect of graded hypoxia and hypercapnia. *Physiol. Biochem. Zool.* **75**, 77-82.

Frappell, P. B. and Mortola, J. P. (1994). Hamsters vs. rats: metabolic and ventilatory response to development in chronic hypoxia. *J. Appl. Physiol.* **77**, 2748-2752.

Frerichs, K. U. and Hallenbeck, J. M. (1998). Hibernation in ground squirrels induces state and species-specific tolerance to hypoxia and aglycemia: an in vitro study in hippocampal slices. *J. Cereb. Blood Flow Metab.* **18**, 168-175.

Frerichs, K. U., Kennedy, C., Sokoloff, L. and Hallenbeck, J. M. (1994). Local cerebral blood flow during hibernation, a model of natural tolerance to "cerebral ischemia". *J. Cereb. Blood Flow Metab.* **14**, 193-205.

Frerichs, K. U., Dienel, G. A., Cruz, N. F., Sokoloff, L. and Hallenbeck, J. M. (1995). Rates of glucose utilization in brain of active and hibernating ground squirrels. *Am. J. Physiol.* **268**, R445-R453.

Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? *J. Comp. Physiol. B* **158**, 25-37.

Gentile, N. T., Spatz, M., Brenner, M., McCarron, R. M. and Hallenbeck, J. M. (1996). Decreased calcium accumulation in isolated nerve endings during hibernation in ground squirrels. *Neurochem. Res.* **21**, 947-954.

Globus, M. Y., Alonso, O., Dietrich, W. D., Busto, R. and Ginsberg,

M. D. (1995). Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J. Neurochem.* **65**, 1704-1711.

Harris, M. B. and Milsom, W. K. (1995). Parasympathetic influence on heart rate in euthermic and hibernating ground squirrels. *J. Exp. Biol.* **198**, 931-937.

Heller, H. C., Colliver, G. W. and Beard, J. (1977). Thermoregulation during entrance into hibernation. *Pflügers Arch.* **369**, 55-59.

Hermes-Lima, M. and Zenteno-Savin, T. (2002). Animal response to drastic changes in oxygen availability and physiological oxidative stress. *Comp. Biochem. Physiol. C* **133**, 537-556.

Hiestand, W. A., Rockhold, W. T., Stemler, F. W., Stullken, D. E. and Wiebers, J. E. (1950). The comparative hypoxic resistance of hibernators and nonhibernators. *Physiol. Zool.* **23**, 264-268.

Holzer, M. and Sterz, F. (2003). Therapeutic hypothermia after cardiopulmonary resuscitation. *Expert Rev. Cardiovasc. Ther.* **1**, 317-325.

Huh, P. W., Belayev, L., Zhao, W., Koch, S., Busto, R. and Ginsberg, M. D. (2000). Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia. *J. Neurosurg.* **92**, 91-99.

Hypothermia after Cardiac Arrest Study Group (2002). Therapeutic hypothermia to improve neurologic outcome after cardiac arrest. *New Engl. J. Med.* **346**, 549-556.

Jackson, D. C. (2002). Hibernating without oxygen: physiological adaptations of the painted turtle. *J. Physiol.* **543**, 731-737.

Johnson, G. L. and Lapadat, R. (2002). Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* **298**, 1911-1912.

Kunz, M., Ibrahim, S., Koczan, D., Thiesen., H. J., Kohler, H. J., Acker, T., Plate, K. H., Ludwig, S., Rapp, U. R., Brocker, E. B. et al. (2001). Activation of c-Jun NH_2-terminal kinase/stress-activated protein kinase (JNK/SAPK) is critical for hypoxia-induced apoptosis of human malignant melanoma. *Cell Growth Differ.* **12**, 137-145.

Le, Y. J. and Corry, P. M. (1999). Hypoxia-induced bFGF gene expression is mediated through the JNK signal transduction pathway. *Mol. Cell Biochem.* **202**, 1-8.

Lee, M., Choi, I. and Park, K. (2002). Activation of stress signaling molecules in bat brain during arousal from hibernation. *J. Neurochem.* **82**, 867-873.

Lust, W. D., Wheaton, A. B., Feussner, G. and Passonneau, J. (1989). Metabolism in the hamster brain during hibernation and arousal. *Brain Res.* **489**, 12-20.

Lutz, P. L. and Nilsson, G. E. (1997). Contrasting strategies for anoxic brain survival – glycolysis up or down. *J. Exp. Biol.* **200**, 411-419.

Lyman, C. P. (1982). Recent theories of hibernation. In *Hibernation and Torpor in Mammals and Birds* (ed. C. P. Lyman, J. S. Willis, A. Malan and L. H. C. Wang), pp. 104-123. New York: Academic Press.

Lyman, C. P. and Chatfield, P. O. (1955). Physiology of hibernation in mammals. *Physiol. Rev.* **35**, 403-425.

Ma, Y. L., Rice, M. E., Chao, M. L., Rivera, P. M., Zhao, H. W. Ross, A. P., Zhu, X., Smith, M. A. and Drew, K. L. (2004). Ascorbate distribution during hibernation is independent of ascorbate redox state. *Free Radic. Biol. Med.* **37**, 511-520.

Maginniss, L. A. and Milsom, W. K. (1994). Effects of hibernation on blood oxygen transport in the golden-mantled ground squirrel. *Respir. Physiol.* **95**, 195-208.

McNamara, M. C. and Riedesel, M. L. (1973). Memory and hibernation in *Citellus literalis*. *Science* **179**, 92-94.

Mihailovic, L. J., Petrovc, B., Protic, S. and Divac, I. (1968). Effects of hibernation on learning and memory. *Nature* **218**, 191-192.

Milsom, W. K., Zimmer, M. B. and Harris, M. B. (1999). Regulation of cardiac rhythm in hibernating mammals. *Comp. Biochem. Physiol. A* **124**, 383-391.

Mortola, J. P. (1991). Hamsters versus rats: ventilatory responses in adult and newborns. *Respir. Physiol.* **85**, 305-317.

Osborne, P. G. and Hashimoto, M. (2003). State-dependent regulation of cortical blood flow and respiration in hamsters: Response to hypercapnia during arousal from hibernation. *J. Physiol.* **547**, 963-970.

Osborne, P. G., Hu, Y., Covey, D. N., Barnes, B. M., Katz, Z. and Drew, K. L. (1999). Determination of striatal extracellular gamma-aminobutyric acid in non-hibernating and hibernating arctic ground squirrels using quantitative microdialysis. *Brain Res.* **839**, 1-6.

Park, S. Y., Lee, H., Hur, J., Kim, S. Y., Kim, H., Park, J. H., Cha, S., Kang, S. S., Cho, G. J., Choi, W. S. et al. (2002). Hypoxia induces nitric oxide production in mouse microglia via p38 mitogen-activated protein kinase pathway. *Mol. Brain Res.* **107**, 9-16.

Perez-Pinzon, M. A., Lutz, P. L., Sick, T. J. and Rosenthal, M. (1997). Metabolic mechanisms of anoxia tolerance in the turtle brain. *Adv. Exp. Med. Biol.* **411**, 75-81.

Popov, V. I. and Bocharova, L. S. (1992). Hibernation-induced structural changes in synaptic contacts between mossy fbres and hippocampal pyramidal neurons. *Neuroscience* **48**, 53-62.

Popov, V. I., Bocharova, L. S. and Bragin, A. G. (1992). Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neuroscience* **48**, 45-51.

Prendergast, B. J., Freeman. D. A., Zucker, I. and Nelson, R. J. (2002). Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **282**, R1054-R1062.

Reis, D. J., Golanov, E. V., Galea, E. and Feinstein, D. L. (1997). Central neurogenic neuroprotection: central neural systems that protect the brain from hypoxia and ischemia. *Ann. N. Y. Acad. Sci.* **835**, 168-186.

Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *J. Neurosci.* **22**, 357-364.

Sidky, Y. A., Hayward, J. S. and Ruth, R. F. (1972). Seasonal variation of the immune response of ground squirrels kept at 22-24°C. *Can. J. Physiol. Pharmacol.* **50**, 203-206.

Spurrier, W. A. and Dawe, A. R. (1973). Several blood and circulatory changes in the hibernation of the 13-lined ground squirrel, *Citellus tridecemlineatus*. *Comp. Biochem. Physiol. A* **44**, 267-282.

Stenzel-Poore, M. P., Stevens, S. L., Xiong, Z., Lessov, N. S., Harrington, C. A., Mori, M., Meller, R., Rosenzweig, H. L., Tobar, E., Shaw, T. E. et al. (2003). Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet* **362**, 1028-1037.

Tahti, H. and Soivio, A. (1975). Blood gas concentrations, acid-base balance and blood pressure in hedgehogs in the active state and in hibernation with periodic respiration. *Ann. Zool. Fennici* **12**, 188-192.

Tattersall, G. J. and Milsom, W. K. (2003). Transient peripheral warming accompanies the hypoxic metabolic response in the golden-mantled ground squirrel. *J. Exp. Biol.* **206**, 33-42.

Tenney, S. M. and Boggs, D. F. (1986). Comparative mammalian respiratory control. In *Handbook of Physiology, Section 3, Respiration, vol. II: Control of Breathing* (ed. A. P. Fishman), pp. 833-855. Bethesda, MD: American Physiological Society.

Toien, O., Drew, K. L., Chao, M. L. and Rice, M. E. (2001). Ascorbate dynamics and oxygen consumption during arousal from hibernation in Arctic ground squirrels. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **281**, R572-R583.

Twente, J. W. and Twente, J. (1978). Autonomic regulation of hibernation by *Citellus* and *Eptesicus*. In *Strategies in the Cold: Natural Torpor and Thermogenesis* (ed. L. Wang and J. W. Hudson), pp. 327-373. New York: Academic Press.

Walsh, J. P., Boggs, D. F. and Kilgore, D. L., Jr (1996). Ventilatory and metabolic responses of a bat, Phyllostomus discolor, to hypoxia and CO2: implications for the allometry of respiratory control. *J. Comp. Physiol. B* **166**, 351-358.

Wang, S. Q., Lakatta, E. G., Cheng, H. and Zhou, Z. Q. (2002). Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. *J. Exp. Biol.* **205**, 2957-2962.

Weibel, E. R. (1999). Understanding the limitation of O_2 supply through comparative physiology. *Respir. Physiol.* **118**, 85-93.

Widmer, H. R., Hoppeler, H., Nevo, E., Taylor, C. R. and Weibel, E. R. (1997). Working underground: respiratory adaptations in the blind mole rat. *Proc. Natl. Acad. Sci. USA* **94**, 2062-2067.

Xiao, J. and Liu, Y. (2003). Differential roles of ERK and JNK in early and late stages of neuritogenesis: a study in a novel PC12 model system. *J. Neurochem.* **86**, 1516-1523.

Zhou, F., Braddock, J. F., Hu, Y., Zhu, X., Castellani, R. J., Smith, M. A. and Drew, K. L. (2001a). Microbial origin of glutamate, hibernation and tissue trauma: an in vivo microdialysis study. *J. Neurosci. Meth.* **119**, 121-128.

Zhou, F., Zhu, X., Castellani, R. J., Stimmelmayr, R., Perry, G., Smith, M. A. and Drew, K. L. (2001b). Hibernation, a model of neuroprotection. *Am. J. Pathol.* **158**, 2145-2151.

Zimmer, M. B. and Milsom, W. K. (2001). Effects of changing ambient temperature on metabolic, heart, and ventilation rates during steady state hibernation in golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiol. Biochem. Zool.* **74**, 714-723.