A new insight into the ability to resist Ischemic brain injury: Does hibernation matter? An Editorial comment for 'Arctic ground squirrel hippocampus tolerates oxygen glucose deprivation independent of hibernation season even when not hibernating and after ATP depletion, acidosis and glutamate efflux'

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Ischemic injury results in tissue hypoxia (reduced O\textsubscript{2}) during which the tissue is not able to obtain adequate O\textsubscript{2}. This results in a fall in the partial pressure of O\textsubscript{2} inside the tissue and a corresponding reduction in mitochondrial respiration and oxidative metabolism. In addition, reduced blood flow restricts delivery of nutrients such as glucose and removal of metabolic waste (Bhowmick et al. 2017). Ischemic brain injury in stroke patients is a global medical issue. More than 200 estimated clinical studies have been implemented, and several pharmacotherapies for ischemic stroke have been developed. None of these has resulted in the development of chemical neuroprotection for ischemic stroke. Tissue plasminogen activator, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. However, tissue plasminogen activator has a limited therapeutic window, and not every ischemic stroke patient is eligible for the therapy. Much can be learned from a model system such as the Arctic ground squirrel (AGS) on how to resist brain damage (Miyake et al. 2015). The Arctic ground squirrel has a unique ability to resist brain damage caused by global cerebral ischemia. This ability is better than other models of tolerance including ischemia preconditioning (Bhowmick et al. 2017). Therefore, the AGS model provides vital information on how to resist brain damage following cerebral ischemia/reperfusion (I/R) (Miyake et al. 2015), caused by cardiac arrest and resuscitation (Jinka et al. 2012).

Along this line of research, analysis of brain mechanisms and unique adaptations to tolerate physiological extremes and subsequently cerebral I/R has become an active field of inquiry with new results and novel insights emerging at a stable pace. A recent study on how AGS hippocampus tolerates oxygen glucose deprivation (OGD) injury (Bhowmick et al. 2017) reveals that the influence of season on cerebral ischemia tolerance is inconsequential in AGS’s ability to resist ischemic injury. Such a study provides insight into a model system with an adaptation to tolerate ischemic injury, and resist brain damage following cerebral ischemia/reperfusion.

Highlight

The advancement in understanding physiological changes during hibernation and active states on ischemia tolerance is thought to offer significant strategies on how to mount a wide range of resistance to prevention and treatment of ischemic...
injury. Moreover, it is also important to determine whether such tolerance persists under conditions that mimic acidosis and the ischemic shifts features of cerebral I/R in vivo. In their article, Bhowmick and his colleagues assess the influence of hibernating or not hibernating on resistance to OGD in AGS brain tissue. In addition, they determined whether the loss of ATP, acidosis and glutamate efflux associated with anoxic depolarization are reduced in an OGD-tolerating AGS. They tested the hypothesis that tolerance to I/R injury modeled in an acute hippocampal slice preparation in AGS is independent of the hibernation season and persists even after glutamate efflux.

Differences between summer AGS and all other AGS were assessed to determine the influence of season on tolerance to cerebral I/R injury. In their study, Bhowmick and his colleagues used AGS that were late in their torpor bout after 80–90% of the duration of the previous torpor bout (8–12 days), and were defined as hibernating AGS. AGS that were early after induced arousal from torpor (4 and 20 h) were defined as interbout euthermic AGS. Post-reproductive summer euthermic animals were collected 2–3 months after ending hibernation, and were used as summer euthermic AGS. As measured by the OGD-induced cell death, they showed that hibernation season has no effect on the ability of the AGS to resist ischemic injury. Moreover, authors reported that tolerance to OGD in AGS hippocampal slices occurs despite loss of ATP, and glutamate release and persists during conditions that mimic acidosis and ionic shifts characteristic of cerebral I/R. Previous studies have shown that following cerebral I/R caused by cardiac arrest or mimicked by in vitro preparations, AGS has the inherent ability to tolerate cerebral ischemia/reperfusion (Dave et al. 2009) and that cerebral I/R initiates cascades of events that facilitates the excitatory release of glutamate that leads to the detrimental effect (Fontana 2015). In their study, Bhowmick and his colleagues designed a series of experiment and determined whether the observed tolerance to cerebral ischemia/reperfusion in the AGS is dependent on the hibernation season (Fig. 1).

Using an improved method to study cell death in adult AGS tissue, Bhowmick and his colleagues used a novel microperfusion approach which isolates processes occurring at the tissue level so that interpretation of the result is not confounded by whole animal physiology. Moreover, the microperfusion approach allows for the concentration of each component in the perfusion fluid to be defined and monitored. They tested the hypothesis that tolerance to I/R injury modeled in an acute hippocampal slice preparation in AGS is independent of the hibernation season and persists even after glutamate efflux.

Fig. 1 A graphic demonstration that Arctic ground squirrel (AGS) hippocampal slices tolerate oxygen glucose deprivation (OGD) injury independent of hibernation season (summer vs. winter) and state (hibernating vs. interbout arousal) based on Bhowmick et al. (2017). AGS is reported to tolerate OGD (shown in green arrow) more than ischemic susceptible Sprague-Dawley rat (shown in red arrow) despite loss of ATP, glutamate release, and acidosis. The study found no difference in tolerance to OGD between winter hibernating and summer active AGS, suggesting that effect of season on cerebral ischemia tolerance is immaterial in AGS. The physiological mechanism that occurs downstream to facilitate glutamate release that enhances tolerance to OGD in AGS hippocampus is not very clear. They propose mechanisms of tolerance at the tissue level that are unique to AGS. We think that this requires further investigation to understand the downstream mechanism that regulates glutamate efflux to facilitate tolerance to OGD in AGS hippocampus, as this may likely produce additional, and possibly more opportunities for therapeutic intervention for ischemic injury. This is a comment on the highlighted study.

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manipulated such that cell death can be quantified in a time-dependent manner. It is noteworthy that the microperfusion approach utilized in this study provides a greater control over the extracellular environment that could not be achieved in vivo. Although the slice preparation differs from the in vivo scenario, it serves their objective to study processes at the tissue level that do not depend on cold tissue temperatures and confer tolerance to AGS.

Moreover, the ability to strictly control the individual components of the perfusion fluid provides the opportunity to decrease the pH without hypoxia; thus cell death can be monitored with temporal resolution relevant to onset and recovery from low pH. The ability to distinctly manipulate individual components of the extracellular setting is not possible in vivo in which pH decreases during anoxia. Therefore, the microperfusion approach used in this study provides an advanced control over the extracellular environment that could not be achieved in vivo.

The first prominent finding is that there was no difference in tolerance to OGD between winter hibernating and summer active AGS. This finding indicates that the influence of season on cerebral ischemia tolerance does not influence AGS’s ability to resist ischemic injury. This study’s finding is consistent with whole animal studies that reveal that tolerance to I/R or hypoxia does not require the hibernation season (Bogren et al. 2014). Authors proposed that AGS does not need to be cold to tolerate cerebral I/R, and that cold tissue temperatures would likely enhance tolerance. Authors did not include ischemic animals’ models in their study, and could not define the influence of the hibernating state in whole animal models as they could not control the effect of different brain tissue temperatures in AGS that are hibernating or awake. They cited whole animal studies that are consistent with their finding that tolerance does not depend on the hibernation season (Bogren et al. 2014), and argued that it is also possible that ischemic tolerance irrespective of season may be unique to the AGS. A recent study in AGS did reveal cardioprotective benefit of the hibernation season during cardiopulmonary bypass in vivo with a core body temperature of 18°C (Quinones et al. 2016). With respect to this finding, Bhowmick and his colleagues argued that it is possible that the tissue response to ischemia differs from heart to brain; i.e. the longer duration of ischemia investigated in the cardiopulmonary bypass procedure may have indicated a seasonal difference not evident in the investigation of cerebral I/R tolerance in AGS. Therapeutic hypothermia has been used in cardiac arrest and stroke surgeries to improve survival, therefore, the potential contribution of this study to clinical application is important.

Bhowmick and colleagues observed that tolerance to OGD in AGS occurs despite loss of ATP and glutamate release. Furthermore, this tolerance persists during conditions that mimic acidosis and ionic shifts characteristic of cerebral I/R. Evidence of neuronal tolerance to a prolonged ischemic shift solution was previously observed after ischemic insult in painted turtle neurons (Pamenter et al. 2012). In line with this finding, Bhowmick and colleagues explained that at the tissue level when tissue temperature is normalized to 36°C despite ATP depletion, ionic derangement, tissue acidosis, and excitatory neurotransmitter efflux, the AGS hippocampus has the capability to resist OGD injury. This suggest an evolution of unique adaptations to physiological extremes and subsequently cerebral I/R. A major conclusion of this study is that tolerance persists independent of hibernation season despite ATP depletion, acidosis, and glutamate efflux. This finding highlights the need for more research to investigate the mechanisms that regulate downstream to glutamate efflux that facilitates tolerance to OGD in AGS hippocampus.

Although this new observation that ischemia tolerance persists independent of hibernation season (despite ATP depletion, acidosis, and glutamate efflux) is crucial, the discovery of a downstream mechanism that regulates glutamate efflux to facilitate tolerance to OGD in AGS hippocampus is likely to produce additional, and possibly more opportunities for therapeutic intervention for ischemic injury. Moreover, as Barhwal et al. (2015) demonstrated that mitochondrial enzyme activity plays a major role in improving ischemic tolerance in hibernating animals, an investigation of the potential contribution of the mitochondrial function to hibernation state (i.e., hibernating or not hibernating) and hibernation season (i.e., winter vs. summer) on resistance to OGD in AGS hippocampal cells would shed further light on this phenomenon.

The work goes on to suggest that resistance to OGD in AGS hippocampus is not as a result of attenuation in excitatory amino acid efflux such as glutamate and aspartate. They pointed to their observation that the 30 min of OGD applied in their study should have been sufficient to depolarize hippocampal neurons and regulate glutamate and aspartate efflux in a manner similar to rats occurring with a time course consistent with in vivo studies. While delayed depolarization may contribute to tolerance, they suggested that an unidentified mechanism of tolerance unique to the AGS may occur to regulate glutamate release that facilitates tolerance to OGD in the AGS hippocampus.

In general, one of the most unique aspects of this study is the investigation of the effect of the hibernating state (hibernating or not hibernating) and hibernation season (winter vs. summer) on resistance to OGD in AGS brain tissue, and whether OGD in AGS hippocampal slices occurs following the loss of ATP, and glutamate release that persists during conditions that mimic acidosis and ionic shifts characteristic of cerebral I/R. An implication of the work is that the AGS has the unique ability to tolerate OGD, and that this tolerance exists under conditions that mimic acidosis and ischemic shifts characteristic of cerebral I/R in vivo. The translational implications of these findings are
very important, as they support the premise that tolerance to ischemic injury in model systems can offer important strategies to prevent a transient or a permanent decrease in blood flow to the brain. Consistent with this possibility, Bhowmick and his colleagues propose that the AGS has a unique ability to resist cerebral ischemia/reperfusion (I/R) that triggers a cascade of uncontrolled cellular processes that perturb cell homeostasis, and cause neuronal damage in the brain of a stroke patient. This uncontrolled cascade of cellular events leads to permanent neurological damage resulting in high rates of disability and death among stroke patients (Ruan et al. 2015). Interestingly, Bhowmick and his colleagues show that the ability of hippocampal neurons of AGS to resists OGD injury is not because of enhanced energy conservation. They reported that energy homeostasis is disrupted in AGS in a manner similar to rats and that tolerance to OGD cannot be explained by improved energy homeostasis. Thus, this study highlights and reinforces the need for more research into not only the mechanism for tolerance to OGD in AGS but also the role of mitochondrial function in ischemic tolerance. The overarching theme is to understand the cellular and molecular mechanisms that transform the cellular network into a neuroprotective state in the brain of the AGS. This could open new and different dimensions for research in ischemic injury during which occlusion of cerebral or penetrating arterial blood supply facilitates either a temporary or an enduring decrease in blood flow to the brain.

Acknowledgments and conflict of interest disclosure

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References


