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Review

Beyond anoxia: The physiology of metabolic downregulation and recovery in the anoxia-tolerant turtle ☆

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Abstract

The freshwater turtle *Trachemys scripta* is among the most anoxia-tolerant of vertebrates, a true facultative anaerobe able to survive without oxygen for days at room temperature to weeks or months during winter hibernation. Our good friend and colleague Peter Lutz devoted nearly 25 years to the study of the physiology of anoxia tolerance in these and other model organisms, promoting not just the basic science but also the idea that understanding the physiology and molecular mechanisms behind anoxia tolerance provides insights into critical survival pathways that may be applicable to the hypoxic/ischemic mammalian brain. Work by Peter and his colleagues focused on the factors which enable the turtle to enter a deep hypometabolic state, including decreases in ion flux ("channel arrest"), increases in inhibitory neuromodulators like adenosine and GABA, and the maintenance of low extracellular levels of excitatory compounds such as dopamine and glutamate. Our attention has recently turned to molecular mechanisms of anoxia tolerance, including the upregulation of such protective factors as heat shock proteins (Hsp72, Hsc73), the reversible downregulation of voltage gated potassium channels, and the modulation of MAP kinase pathways. In this review we discuss three phases of anoxia tolerance, including the initial metabolic downregulation over the first several hours, the long-term maintenance of neuronal function over days to weeks of anoxia, and finally recovery upon reoxygenation, with necessary defenses against reactive oxygen stress.

Keywords: Anoxia; Antioxidants; Channel arrest; Hypometabolism; Neurotransmitter; Reactive oxygen species; *Trachemys scripta*

Contents

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1. Introduction

Oxygen is considered critical to nearly all life on earth, as the end electron acceptor that makes mitochondrial oxidative phosphorylation possible. Acute hypoxia or cerebral ischemia causes neuronal death due to the failure of ATP-driven ion transporters; the breakdown of membrane potential is followed by the release of excitatory amino acids (EAA) such as aspartate and glutamate, with glutamate binding to postsynaptic receptors that regulate calcium channels. The resulting Ca^{2+} influx activates proteases, lipases, and endonucleases, which in turn destroy cellular integrity. Additional neuronal damage occurs during reperfusion, thought to be caused by the post-ischemic release of oxygen radicals, the synthesis of nitric oxide, inflammation, and an imbalance between the excitatory and inhibitory neurotransmitter systems (Berger et al., 2002). When temperature differences are taken into account these catastrophic effects of hypoxia/ischemia are characteristic of the brains of nearly all vertebrates ranging from fish to mammals (Lutz et al., 2003a,b). Neurons are generally viewed as among the most anoxia sensitive of all cells, although recent studies have shown a wide variation in the capacity of neurons to tolerate hypoxia, reflective of function and the degree of hypoxia normally encountered.

Not all animals, however, share the mammalian susceptibility to hypoxia, or even full anoxia. Freshwater turtles of the genera *Trachemys* and *Chrysemys* are true facultative anaerobes, able to survive from up to 48 h at room temperature to months (during winter hibernation) in the total absence of oxygen (Jackson, 2000). *Trachemys scripta* has been the subject of extensive research into the adaptations that permit neuronal survival without oxygen; the turtle is able to decrease its metabolic rate to approximately 10–15% of basal, such that energy utilization is matched to anaerobic energy production (Lutz et al., 2003a,b). By preventing an energy deficit, the turtle brain avoids the catastrophic drop in ATP levels which in mammalian neurons results in the breakdown of cellular ion homeostasis, release of excitatory neurotransmitters, and excitotoxic cellular death (Lutz et al., 2003a,b). To decrease neuronal energy requirements, *Trachemys* decreases membrane ion permeability ("channel arrest") (Chih et al., 1989), inhibits the release of excitatory neurotransmitters such as dopamine (Milton and Lutz, 1998) and glutamate (Milton et al., 2002), increases the release of inhibitory compounds such as adenosine (Nilsson and Lutz, 1992) and GABA (Nilsson and Lutz, 1991), and decreases electrical activity (Fernandes et al., 1997). Thus at no time do turtle neurons experience an energy deficit that would otherwise constitute a trigger for catastrophic cell death. Such extended anoxic survival time is not a matter of ectothermy, as other reptiles survive only 20–30 min without oxygen (Belkin, 1963) and do not exhibit the same neurological adaptations that permit true anoxic tolerance (Nilsson et al., 1991).

A very extensive body of knowledge concerning the mechanisms of anoxia tolerance was contributed by our good friend and colleague, Peter Lutz, who was a pioneer and leader in this field. Peter authored more than 50 articles in his lifetime on anoxia tolerance alone, within a career of interests ranging from Nigerian trematodes (Lutz and Siddiqi, 1967), to the platypus (Lutz et al., 1989). Moving to South Florida in 1976, he soon began a series of studies on the diving physiology of sea turtles; their remarkable ability to dive for long periods of time soon piqued Peter's interest in brain survival during hypoxia, and this in turn led him to begin investigating the best of facultative anaerobes, the freshwater turtle. This interest continued and expanded from his first *Trachemys* paper in 1980 until his death in 2005. This review will cover many of those significant findings, along with some directions we expect to more fully investigate in the future, building on the legacy of scientific inquiry among the many friends, students, and colleagues who continue working in this field.

It became clear in recent years that while a great deal of effort has gone into describing the events leading from normoxic physiology to anoxic hypometabolism, there are three significant phases of anoxic survival, each with its own challenges. To survive bouts of anoxia the turtle brain must be capable of (1) tolerating an early transition to anoxia (the first 1–2 h), (2) a more prolonged deep hypometabolic state where neuronal network integrity must be maintained (to permit recovery at a later time when oxygen becomes available) and finally (3) a re-oxygenation phase where the turtle brain must contend with the potential for massive levels of reactive oxygen species while simultaneously restoring normal brain function.