Comparative Biochemistry and Physiology, Part B xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

The hypoxia-tolerant vertebrate brain: Arresting synaptic activity

Leslie T. Buck[a,b,*], Matthew E. Pamenter[c,d]

[a] Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada
[b] Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
[c] Department of Biology, University of Ottawa, Ottawa, ON, Canada
[d] University of Ottawa, Brain and Mind Research Institute, Canada

ARTICLE INFO

Keywords:
Ion channel arrest
Anoxia
Neurotransmission
Turtle
Goldfish
Naked mole rat
Squirrel

ABSTRACT

The ion channel arrest hypothesis has been the foundation of three decades of research into the underlying mechanisms of hypoxia/anoxia tolerance in several key species, including: painted turtles, goldfish, crucian carp, naked mole rats, and arctic and ground squirrels. The hypothesis originally stated that hypoxia/anoxia tolerant species ought to have fewer ion channels per area membrane and/or mechanisms to regulate the conductance of ion channels. Today we can add to this and include mechanisms to remove channels from membranes and the expression of low conductance isoforms. Furthermore, possible oxygen sensing mechanisms in brain include a link to mitochondrial function, changes in the concentration of intracellular Ca^{2+} and reactive oxygen species, and activation of protein kinase C and a phosphatase. Importantly ion channel arrest leads to a decrease in metabolic rate that is fundamental to survival without oxygen and in brain is reflected in decreased action potential frequency or spike arrest. This results not only from a decrease in excitatory glutamatergic receptor currents but also by an increase in inhibitory GABAergic receptor currents. The surprising finding that ionic conductance through some ion channels increases is novel and contrary to the ion channel arrest hypothesis. The major insight that this offers is that key regulatory events are occurring at the level of the synapse and we therefore propose the "synaptic arrest hypothesis".

1. Introduction

Just over 30 years ago Peter Hochachka published his seminal paper outlining his metabolic and ion channel arrest hypotheses (Hochachka, 1986). In it he describes the decoupling of ATP production from ATP demand during periods of anoxia and/or hypothermia as a fundamental challenge for the majority of vertebrates faced with an oxygen shortage. Since glycolysis can only produce about a tenth of the ATP that oxidative phosphorylation does and increasing the glycolytic rate only leads to the rapid depletion of glucose stores and production of toxic endproducts (H^+, lactate), he reasoned that ATP demand must decrease to meet decreased supply to survive severe hypoxia. He was aware that maintaining ion gradients across cell membranes was an energetically expensive process and that stabilizing ion gradients during hypoxia was protective (Lutz et al., 1985; Hochachka, 1985); therefore, he hypothesized that anoxia tolerant species had either a lower density of ion channels per area membrane or that ion channel permeability changed with the onset of anoxia which resulted in decreased demand for ATP by ion motive pumps. Although mostly associated with ectothermy it did appear that anoxia-tolerant species had plasma membranes that were less leaky to ions (Else and Hulbert, 1987; Xia and Haddad, 1991). Interestingly, 2 years after he proposed ion channel arrest as a means to survive hypoxic/anoxic periods an oxygen sensing K^+ channel was reported, but the means by which this channel sensed oxygen is still not agreed upon (López-Barneo et al., 1988). As yet there is no evidence indicating that ion channel density changes in the short term but there are many examples of ion channel gating properties changing acutely with hypoxic exposure (López-Barneo et al., 1988; Shimoda and Polak, 2011). However, changes in the number of functional ion channels over long-term hypoxia have been observed and may be considered a third aspect of the ion channel arrest hypothesis (Bickler et al., 2000; Perez-Pinzon et al., 1992; Lutz and Leonekabler, 1995).

Another addition to channel arrest could be the expression of lower conductance forms of ion channels which will be discussed later.

Abbreviations: AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid; AP, action potential; AP_{freq}, AP frequency; ATP, Adenosine triphosphate; $[Ca^{2+}]$, Calcium concentration; ECD, excitotoxic cell death; FCCP, Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; GABA, γ-Amino-butyric acid; mK_{ATP}, mitochondrial ATP-sensitive potassium channel; mK_{Ca}, Mitochondrial calcium-sensitive potassium channel; MOMP, mitochondrial outer membrane permeabilization; MPTP, mitochondrial permeability transition pore; NMDA, *N*-methyl-D-aspartate; OGD, oxygen glucose deprivation; PKC, protein kinase C; Vm, membrane potential

* Corresponding author at: Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada.
E-mail address: les.buck@utoronto.ca (L.T. Buck).

https://doi.org/10.1016/j.cbpb.2017.11.015
Received 15 September 2017; Received in revised form 23 November 2017; Accepted 30 November 2017

Although there is considerable evidence pointing to which aspects of cellular metabolism are regulated (Bickler and Buck, 2007; Staples and Buck, 2009; Krivoruchko and Storey, 2015), the coordinated mechanisms that regulate a transition from an oxidative to a non-oxidative state are still unknown. In this review we will focus mostly on neuronal mechanisms of hypoxia tolerance in several hypoxia and anoxia tolerant vertebrates including; freshwater turtle, goldfish, crucian carp, naked mole rat, mole rat (Spalax) and the arctic ground squirrel. As will be discussed, the ion channel arrest hypothesis does not fully encompass what occurs in anoxia-tolerant brain; therefore, we propose the term "synaptic arrest hypothesis" to describe mechanisms that occur at the synapse to regulate energetically expensive ion movement during periods of low oxygen availability.

1.1. Hypoxia is a pathological and environmental stress

Hypoxia is a key component of many pathological conditions, including heart attack, stroke, chronic pulmonary disorders, severe asthma, hypotension, and altitude-related illnesses, among others. In fact, the brain of most vertebrates cannot tolerate more than a few minutes of hypoxia before suffering neurological impairment and cell death. Beyond pathologies related to reduced oxygen availability, hypoxic environments are also common in nature. For example, hypoxia is a life-long environmental challenge for species that overwinter under ice covered water, inhabit densely packed underground burrows, or inhabit high altitude niches that experience hypobaric hypoxia, among others. For such species, hypoxia has acted as a strong selective force that has driven the evolution of cellular and physiological traits that enhance tolerance to low oxygen stress. Physiological adaptations to life in hypoxia have been recently reviewed (Dzal et al., 2015), and so for the purposes of this review we will focus on cell-level and synapse-level adaptations in the most hypoxia-sensitive organ: the brain. Indeed, the brain of many vertebrates that dwell in hypoxic environments – such as freshwater turtles and fish, hibernating squirrels, and African mole rats – are remarkably tolerant to prolonged hypoxia, and in some cases even anoxia (Bickler and Buck, 2007). The characteristic loss of ionic homeostasis associated with hypoxic injury in brain is not seen in these species, which instead evolved hypoxic defense mechanisms (Buck and Pamenter, 2006; Pamenter, 2014). Remarkably, these mechanisms confer protection not only against naturally occurring environmental (and thus systemic) hypoxia, but also clinically relevant ischemic models of stroke such as oxygen glucose deprivation (OGD).

1.2. Hypoxia is excitotoxic to the brain cells of most mammals

Glutamate receptors play a central role in memory formation; however, deranged glutamatergic signaling also plays a key role in the pathology of brain cell death during low oxygen stress (e.g. hypoxia, anoxia, ischemia). Indeed, the brain of most mammals is acutely sensitive to hypoxia or ischemia and rapidly undergoes excitotoxic cell death (ECD) (Choi, 1994; Lundberg and Oscarsson, 1953). Specifically, in the brain of hypoxia-intolerant organisms, low oxygen stress induces elevations in the excitatory amino acid glutamate (Abele et al., 1990; Andine et al., 1991; Bosley et al., 1983), primarily by the reversed operation of glutamate transporters (Rossi et al., 2000). Accumulation of glutamate in the synaptic cleft chronically activates glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and *N*-methyl-D-aspartate (NMDA) receptors, which permit excessive Na^+ and Ca^{2+} influx and consequentially leads to neuronal depolarization and electrical hyper-excitability (Abele et al., 1990; Crepel et al., 1993; Lyubkin et al., 1997; Michaels and Rothman, 1990).

With prolonged low oxygen stress, seizure-like bursts of synaptic activity become more frequent. These excitatory events permit significant ion movement that requires compensation by ATP-dependent pump activity to restore ionic gradients and neuronal homeostasis. However, the hypoxic or ischemic cell is typically unable to utilize oxidative phosphorylation to generate ATP in the absence of oxygen and instead must rely on anaerobic glycolytic pathways of energy production during hypoxia. Glycolytic pathways typically yield only 1/10th of the energy produced by aerobic pathways, and thus the hypoxic or ischemic brain cell suffers an approximate 90% reduction in ATP availability. This energy production deficit, combined with greatly increased ATP demand due to heightened neuronal excitability, results in a rapid depletion of cellular ATP stores (Kopp et al., 1984; Santos et al., 1996). ATP-dependent pumps fail in the absence of ATP and this triggers a further depolarization of neuronal membrane potential (Vm), which rapidly reaches a point of no return, becoming irreversible, even upon reoxygenation (Anderson et al., 2005; Lundberg and Oscarsson, 1953). Extended neuronal depolarization chronically over-activates voltage-sensitive channels and deleterious concentrations of Ca^{2+} and Na^+ continue to enter the cell, leading to further depolarization and acceleration of excitatory events. As the cytosolic [Ca^{2+}] rises, mitochondria take up free Ca^{2+} and mitochondrial [Ca^{2+}] increases concomitantly, opposed by the activity of the mitochondrial Ca^{2+}/H^+ or Ca^{2+}/Na^+ exchangers (Pizzo et al., 2012). When cytosolic [Ca^{2+}] reaches approximately 400–500 nM, the ability of these exchangers to oppose ion gradient-dependent mitochondrial Ca^{2+} uptake is overwhelmed and the mitochondrial [Ca^{2+}] begins to rise rapidly. This is termed the 'set point' and the mitochondrial [Ca^{2+}] becomes overloaded at 1–3 μM cytosolic [Ca^{2+}], although this threshold is modulated by a variety of cellular factors, most notably cellular and mitochondrial pH (Di Lisa and Bernardi, 2009).

Excessive uptake of Ca^{2+} into the mitochondria induces the formation of the mitochondrial permeability transition pore (MPTP), which is a junctional complex that permits ions and solutes up to 1500 Da in size to readily pass out of the mitochondrion and which also enables the release of mitochondrial apoptotic factors that trigger local cell death pathways in neighboring cells (Kannurpatti et al., 2004; Wang and Qin, 2010). Many labs have shown that prevention of MPTP formation is critical to avoiding neuronal apoptosis and necrosis following hypoxic or ischemic damage. For example, in neonatal rat myocytes, ischemia-reperfusion results in apoptotic events that are abolished by cyclosporine A, an inhibitor of the MPTP (Xu et al., 2001). There is some variability in the stage of a given stressor that induces MPTP formation, and in some pathologies and tissues MPTP formation is induced upon reoxygenation (e.g. in cardiomyocytes, Assaly et al., 2012), while in others MPTP formation can occur within minutes of stress onset (e.g. in somatosensory cortex, Liu and Murphy, 2009).

In response to hypoxia or ischemia, most mammalian neurons undergo intrinsic (mitochondrial) apoptotic cell death, mediated by cytochrome *c* release from mitochondria following mitochondrial Ca^{2+} overload (McClintock et al., 2002). Briefly, MPTP formation leads to mitochondrial outer membrane permeabilization (MOMP) via alterations in the balance of the pro- and anti-apoptotic members of the B cell lymphoma 2 (Bcl-2) protein family (Chipuk and Green, 2008). MOMP permits several mitochondrial components to be released into the cytoplasm where they act as pro-apoptotic intracellular signaling molecules to execute programmed cell death. For example, cytochrome *c*, a key component of electron transport, also activates caspase 9-dependent cell death when released into the cytoplasm (Liu et al., 1996). Similarly, apoptosis inducing factor, which is a component of complex I of the electron transport system, acts directly on the nucleus to induce chromatin condensation and fragmentation of the nuclear envelope in caspase-independent cell death (Susin et al., 1999). Beyond the local cell, release of these factors into the surrounding perfusate also induces apoptotic events in neighboring cells, which is a key component of penumbral spread in stroke pathology (Lo, 2008). Considerable research has extensively described cell death pathways related to hypoxic and ischemic cell death and this topic has been expertly reviewed elsewhere (Kroemer et al., 2007; Tait and Green, 2010).

Preventing such rampant excitotoxicity and the execution of cell death pathways are hallmarks of the brain of hypoxia/anoxia-tolerant