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The "Curious Case of Postictal Oxygen Dissipation": Could ROS Be the Culprit?

Postictal Hypoxia Involves Reactive Oxygen Species and Is Ameliorated by Chronic Mitochondrial Uncoupling

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Prolonged severe hypoxia follows brief seizures and represents a mechanism underlying several negative postictal manifestations without interventions. Approximately 50% of the postictal hypoxia phenomenon can be accounted for by arteriole vasoconstriction. What accounts for the rest of the drop in unbound oxygen is unclear. Here, we determined the effect of pharmacological modulation of mitochondrial function on tissue oxygenation in the hippocampus of rats after repeatedly evoked seizures. Rats were treated with mitochondrial uncoupler 2,4 dinitrophenol (DNP) or antioxidants. Oxygen profiles were recorded using a chronically implanted oxygen-sensing probe, before, during, and after seizure induction. Mitochondrial function and redox tone were measured using in vitro mitochondrial assays and immunohistochemistry. Postictal cognitive impairment was assessed using the novel object recognition task. Mild mitochondrial uncoupling by DNP raised hippocampal oxygen tension and ameliorated postictal hypoxia. Chronic DNP also lowered mitochondrial oxygen-derived reactive species and oxidative stress in the hippocampus during postictal hypoxia. Uncoupling the mitochondria exerts therapeutic benefits on postictal cognitive dysfunction. Finally, antioxidants do not affect postictal hypoxia, but protect the brain from associated cognitive deficits. We provided evidence for a metabolic component of the prolonged oxygen deprivation that follow seizures and its pathological sequelae. Furthermore, we identified a molecular underpinning of this metabolic component, which involves excessive oxygen conversion into reactive species. Mild mitochondrial uncoupling may be a potential therapeutic strategy to treat the postictal state where seizure control is absent or poor.

Commentary

The presence of postictal hypoxia may be a key factor contributing to negative consequences of seizures.[1] Postictal hypoxia commonly occurs following seizures and arises from an imbalance in oxygen supply and demand in brain tissue. For example, oxygen supply can be affected by local vasoconstriction that limits blood flow to ictal and postictal regions with a higher metabolic demand.[2] Severe or prolonged postictal hypoxia can facilitate long-term comorbidities including cognitive dysfunction and increased mortality.[3,4] Importantly, both seizures and bouts of hypoxia can have acute and lasting effects on metabolic function and the production of reactive oxygen species (ROS).[5,6]

Mitochondrial oxygen consumption used in oxidative phosphorylation is normally the primary and most efficient means of generating cellular energy. In addition to cellular energy production, the functions of mitochondria are multifaceted and include calcium homeostasis, synthesis of phospholipids and heme, production of ROS, cell cycle control, and orchestration of cell death. Mitochondrial ROS production occurs mainly at complexes I, II, and III, and participates in retrograde redox signaling. Further, ROS production is implicated in mitochondrial dysfunction, pathological oxidative damage, and cellular injury at unabated steady-state levels. The flux of ROS (particularly superoxide) in the mitochondria largely depends upon local oxygen concentration, proton motive force, and the concentration of potential electron donors. Recurrent seizure activity is known to induce ROS production from the mitochondria, and non-mitochondrial systems such as nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase, and xanthine oxidase. Alterations in redox homeostasis can influence energy and cellular metabolism which in turn can perpetuate seizure activity and contribute to epilepsy development.[7,8] During and following seizures, neuronal glucose utilization and overall metabolic activity are increased and may be followed by a hypometabolic phase.[9] Complicating matters, the postictal and interictal periods may also be associated with regional and cellular

differences in metabolism. Therefore, energy use following seizures is multifactorial and complex but also offers opportunities for therapeutic intervention to reduce seizures and comorbidities.

Diminished mitochondrial respiration can divert electrons to the formation of ROS. In tandem with increased postictal intracellular calcium, ROS production can be maintained for longer durations following seizures.[7] Villa et al used a rat kindling model to better understand the relationship between seizure-induced hypoxia and changes in metabolism.[10] Induced seizures in the rat hippocampal kindling model cause local hypoxia that may persist for longer than an hour.[1] This model is useful for the controlled production of seizures, allowing for evaluation of postictal hypoxia and other measurements efficiently. Although induced and spontaneous seizures may lead to differing results, it is noteworthy that hypoxia following kindled and spontaneous seizures are similar.[1] Insights from this study shed light on the interplay between postictal hypoxia and metabolic dysfunction and may facilitate the development of novel therapeutics that restore homeostatic brain metabolism.

The authors used hippocampal mitochondria isolated from kindled rats 40 minutes after the last seizure, a period of long hypoxic exposure determined from previous studies, to evaluate bioenergetics, calcium handling, and the production of ROS. In the basal state, kindled rats show reduced cellular respiration and calcium retention. Furthermore, a mitochondrial membrane permeability transition (MPT) threshold shift in kindled rats demonstrates a critical shift in calcium homeostasis. Oxidative stress, measured using inducible nitric oxide synthase and lipid peroxidation markers, was increased in multiple hippocampal subregions. To counteract these changes, the authors employed chronic ($\sim$24 days) treatment with 30 mg/kg 2,4 dinitrophenol (DNP), which facilitates mild "mitochondrial uncoupling" without influencing/disrupting thermogenic homeostasis. Whereas seizure induction produced hypoxia, DNP treatment led to normoxia and a diminished postictal hypoxic response in the hippocampal tissue. Furthermore, DNP treatment increased calcium retention, raised MPT, reduced ROS production, and reduced lipid peroxidation. While this study clearly demonstrated a hypoxic phenotype in the hippocampal tissue 40 minutes after the last seizure, it was unclear whether the tissue was also ischemic within the time period surrounding mitochondrial isolation and collection. This is an important measure since ROS can be generated from ischemia-reperfusion by reverse electron transport chain and could have contributed to the overall oxidative burden. The assessment of indices such as mitochondrial glutathione levels and superoxide dismutase activity may be beneficial in future studies to accurately and reliably evaluate mitochondrial ROS production and oxidative stress. In this study, the release of hydrogen peroxide (H_2O_2) from isolated mitochondria was measured by using the fluorescence-based Amplex Red assay. Although appropriate controls were incorporated, this assay has certain inherent limitations such as photooxidation of the Amplex Red reagent which can give rise to artifactual ROS production and confound the results obtained. Highly sensitive and specific techniques such as high-performance liquid chromatography and mass spectrometry can be employed for the reliable and holistic measurement of mitochondrial ROS.

The use of DNP, which blocked to some extent the majority of deleterious effects of postictal hippocampal hypoxia, demonstrates the therapeutic potential for mitochondrial uncoupling. Of note, however, is that DNP's clinical use is limited owing to the presence of deleterious cardiovascular, gastrointestinal, and neurological side effects. Some of these adverse effects could be attributed to a heightened metabolic rate and failure of thermoregulatory homeostasis which may occur due to energy being dissipated as heat instead of getting converted to adenosine triphosphate (ATP). The therapeutic benefits and potential risks associated with DNP's mild mitochondrial uncoupling needs to be evaluated carefully in different preclinical animal seizure models to warrant its transition to the clinic.

Nevertheless, this study has demonstrated the potential benefits of mild mitochondrial uncoupling facilitated by DNP treatment. This encompasses the improvement in postictal metabolic outcomes such as the (i) restoration of basal mitochondrial respiration rate and the MPT threshold for calcium buffering, (ii) reduction of mitochondrial ROS production and subsequent oxidative damage to cellular macromolecules, (iii) decrease in ROS-mediated vasoconstriction which preserved hippocampal tissue oxygenation and ameliorated postictal hypoxia, and finally (iv) attenuation of ROS-mediated cognitive impairment. However, as the authors cite a role for vasoconstriction in producing postictal hypoxia, it is unclear the extent to which seizure-dependent shifts in metabolism are fundamentally separated from vasoconstrictive effects. While the authors initially suggest that metabolic dysfunction may represent a potentially separate mechanism, these processes are likely intertwined. Additional studies that tease apart the contributions of cyclooxygenase-2 (COX2) and ROS-mediated vasoconstriction to postictal hypoxia and metabolic impairments are also warranted.

An indirect mechanism by which DNP improves outcomes from postictal hypoxia is plausibly through antioxidant effects. Mild mitochondrial uncoupling significantly reduced tissue hypoxia and decreased H2O2 production which in turn could have prevented further ROS production, lipid peroxidation (formation of 4-hydroxynonenal [4-HNE]), cellular damage, and inflammation, all of which are known to contribute to cognitive impairment. Intriguingly, treatment with either a single antioxidant (N-acetyl cysteine amide [NACA, 150 mg/kg]) or a combination of antioxidants (N-acetyl cysteine [NAC, 500 mg/kg] and sulforaphane [SFN, 5 mg/kg]) was insufficient to prevent hypoxia and decrease H2O2 production. However, it was sufficient to reduce cognitive deficits evaluated in the novel object recognition test. One plausible reason for such paradoxical results could be due to the mechanism of action of these antioxidants: NACA, NAC, and SFN increase cellular glutathione levels which could preferentially scavenge 4-HNE, and hydroperoxides that are products of lipid peroxidation.

However, this increase in cellular antioxidant capacity may not be sufficient to attenuate on-going postictal hypoxia-mediated ROS production, that is, the fraction of oxygen being converted to ROS. Another factor to consider is the treatment duration for DNP compared to the antioxidants. Rats received DNP treatment before, during, and after kindling, whereas antioxidant treatments were initiated predominantly after or toward the end of kindling. This could significantly influence how effectively these treatments could ameliorate on-going ROS production and oxidative stress. The current study offers some explanation for such intriguing results, but future studies that address these discrepancies are warranted.

Overall, chronic mitochondrial uncoupling could be a potential mechanism by which postictal hypoxia-mediated adverse effects (molecular and phenotypic) could be attenuated. Careful evaluation of this treatment strategy is required to fully understand and leverage its therapeutic benefits for the treatment of epilepsy and seizure disorders.

Ashwini Sri Hari, PhD
Department of Pharmacology & Toxicology, University of Utah
Cameron S. Metcalf, PhD
Department of Pharmacology & Toxicology, University of Utah

ORCID iD

Cameron S. Metcalf https://orcid.org/0000-0002-1510-0405

Declaration of Conflicting Interests

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