

THE EMERGING NEUROPROTECTIVE ROLE OF MITOCHONDRIAL UNCOUPLING PROTEIN-2 IN TRAUMATIC BRAIN INJURY

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Abstract

Traumatic brain injury (TBI) is a multifaceted disease with intrinsically complex heterogeneity and remains a significant clinical challenge to manage. TBI model systems have demonstrated many mechanisms that contribute to brain parenchymal cell death, including glutamate and calcium toxicity, oxidative stress, inflammation, and mitochondrial dysfunction. Mitochondria are critically regulated by uncoupling proteins (UCP), which allow protons to leak back into the matrix and thus reduce the mitochondrial membrane potential by dissipating the proton motive force. This uncoupling of oxidative phosphorylation from adenosine triphosphate (ATP) synthesis is potentially critical for protection against cellular injury as a result of TBI and stroke. A greater understanding of the underlying mechanism or mechanisms by which uncoupling protein-2 (UCP2) functions to maintain or optimize mitochondrial function, and the conditions which precipitate the failure of these mechanisms, would inform future research and treatment strategies. We posit that UCP2-mediated function underlies the physiological response to neuronal stress associated with traumatic and ischemic injury and that clinical development of UCP2-targeted treatment would significantly impact these patient populations. With a focus on clinical relevance in TBI, we synthesize current knowledge concerning UCP2 and its potential neuroprotective role and apply this body of knowledge to current and potential treatment modalities.

Keywords

• Neuronal injury • Mitochondria • Traumatic brain injury • Ischemia • Neuronal cell death

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Introduction

Traumatic brain injury (TBI) is a global public health epidemic resulting in over 2.8 million hospitalizations of Americans each year, with more than 500,000 deaths. Because of the composite and destructive effects of both the initial mechanical insult and the secondary injury arising from a variety of biochemical cascades, TBI is a multifaceted disease with intrinsically complex heterogeneity and remains a significant clinical challenge to manage. Experimental TBI models have demonstrated many mechanisms that contribute to neuronal and glial cell death, including glutamate and calcium toxicity, oxidative stress, inflammation, and mitochondrial dysfunction. Mitochondria have been recently established as the principal mediators of cell death, orchestrating the necrotic, apoptotic, and autophagic cellular death pathways [1, 2]. These pathways are critically regulated by uncoupling proteins (UCP), which reduce the mitochondrial membrane potential by causing a proton leak back into the matrix. This process dissipates the proton motive force and uncouples

oxidative phosphorylation from adenosine triphosphate (ATP) synthesis [3]. Of the five UCP thus far discovered, emerging evidence identifies uncoupling protein-2 (UCP2) as potentially critical for protection against cellular injury from TBI and stroke [4-8]. With a focus on clinical relevance in TBI, this review article will synthesize the state of our present knowledge concerning UCP2 and its potential neuroprotective role.

Mitochondria


Mitochondrial ATP synthase completes the enzymatic process of oxidative phosphorylation, converting nutrient energy to reduced cofactors (nicotinamide adenine dinucleotide, NADH, and flavin adenine dinucleotide, FADH₂) through electron shuttling and a proton motive force (PMF) across the inner mitochondrial membrane. These gradient forces consist of a chemical potential and, more critically, an electrical potential ($\Delta\Psi_m$), which accounts for 90% of the PMF [9-12]. This $\Delta\Psi_m$ is the potential energy source that drives phosphorylation of adenosine diphosphate (ADP) to ATP by

allowing hydrogen ions to move down their energy gradient through ATP synthase, but is also the source of potentially dangerous reactive oxygen species (ROS) [13, 14]. In the instance of TBI and stroke, $\Delta\Psi_m$ is too great, resulting in calcium flows into mitochondria with concurrent production of ROS and subsequent cellular damage, thus initiating the process of cell death. This process occurs through the direct result of the $\Delta\Psi_m$ increase triggering the mitochondrial pore transition protein (mPTP) complex [15-18]. An alternative pathway capable of dissipating $\Delta\Psi_m$ to reduce mitochondrial calcium load and mPTP activation without increasing oxidative damage is through the function of uncoupling proteins such as UCP2, which account for 95% of all proton movement that bypasses the ATP synthase [19]. These proteins are ubiquitous enough to account for 25% of the standard metabolic rate [20].

UCP2

UCP1, or thermogenin, was discovered in brown adipose tissue where its diversion of metabolic energy to non-shivering thermal

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energy is recognized for its importance in thermoregulation of newborns as well as in cold-blooded, hibernating, and overfed members of the animal kingdom [21-23]. Discovered in 1997, UCP2 [24] is the best-studied of the non-adipose UCP. UCP2 was well characterized in secondary and tertiary structure in comparison to a bovine ADP/ATP carrier, adenine nucleotide translocase-1 (ANT1), which also permits dissipation of the mitochondrial proton gradient. These two proteins were found to have similar molecular structure despite having only 20% sequence homology [24, 25]. Interestingly, UCP2 appears to have an unusually short half-life of 30 min, compared to its homologue UCP1, which has a half-life of 30 h [26]. This short half-life permits rapid variability of activity at both the mRNA and protein levels [20], suggesting a role in rapid physiological stress responses. UCP2 exists in a glutathione-bound inactive state such that UCP2 activation to reduce ROS production in a stressed cell also liberates a stoichiometric amount of glutathione to mitigate existing ROS moieties [27]. In the central nervous system, mammalian UCP2 mRNA and protein expression is highest in areas that could be described as high-risk for stress. High risk regions would be those with direct access to the blood stream, axons and axon terminals abundant in the hypothalamus, neural circuits important for endocrine and homeostatic regulation [28-30], and those associated with neurodegenerative disease such as the substantia nigra and locus coeruleus [29-32]. UCP2 is recognized as a factor responsible for increased neuronal survival in stressed states such as after stroke or trauma [33-35] through several mechanisms which coalesce at the dissipation and subsequent stabilization of mitochondrial $\Delta\Psi_m$ [31, 32, 36-39], as has been confirmed in transfected cell culture [39].

Physiology of UCP2

Oxidative phosphorylation

UCP dissociate oxidation from phosphorylation, suppressing ATP production per mitochondrion under physiological conditions. During normal cellular function this bypassing of ATP synthase would result in a dangerous and wasteful

futile cycle, but under conditions in which ATP production is limited due to lack of necessary substrates, including oxygen, the dissipation of $\Delta\Psi_m$ through other means would be necessary to protect against ROS damage and initiation of apoptosis [19, 40, 41]. Experimental data suggest that UCP2 contributes to neuronal stress tolerance in acute settings, which over time triggers mitochondrial proliferation and increased ATP/ADP production [32, 42]. As $\Delta\Psi_m$ is intimately linked to ATP production, a disruption of delivery or ability to effectively utilize substrates for oxidative phosphorylation would lead to $\Delta\Psi_m$ increasing to dangerous levels with subsequent ROS production, calcium influx into mitochondria and eventually cell death [39, 40, 43-45]. Recent evidence further indicates that the principle function of UCP2 is to dissipate $\Delta\Psi_m$ *in vivo* [46], which may underlie the emerging neuroprotective role of UCP2 in TBI.

Calcium regulation and apoptosis

Mitochondria represent a point of integration for cell death signaling in vertebrates through the activation of mitochondrial membrane permeabilization [1, 40, 44, 47, 48], releasing myriad cell death factors associated with apoptosis, necrosis, or the recently described hybrid necroptosis [45], and host both pro-survival and pro-apoptotic factors critical for regulating mitochondrial membrane permeability [49]. Regulation of calcium, particularly the shifting of calcium load into mitochondria, is intimately linked to both the necrotic and apoptotic cell death pathways [45, 50]. UCP2 has been identified as a regulator of calcium homeostasis, whose role is best appreciated in cases of cellular stress [51, 52]. Calcium influx into mitochondria increases with a greater $\Delta\Psi_m$, as does ROS production [19, 41]. Thus UCP2 overexpression resulted in decreased $\Delta\Psi_m$ restricting calcium influx into mitochondria and decreased ROS production [39, 43]. The activation of and interaction between several mitochondrial channels, including calcium channels, activated directly or indirectly through mitochondrial $\Delta\Psi_m$, changes under the control of UCP2 and emphasizes the importance of the uncoupler [40, 44, 48].

Reactive oxygen species (ROS)

Reactive oxygen species (ROS), both radicals and non-radicals (hydrogen peroxide), are highly reactive byproducts of oxidative respiration present in all cells that function as signaling elements at physiological concentrations [19, 53]. Mitochondrial ROS production is highly sensitive to slight changes in $\Delta\Psi_m$ [54-56]. UCP2 lowers $\Delta\Psi_m$ with the subsequent decrease of ROS production [38, 42, 57, 58]; conversely, in UCP2 knockout animals, an increase in $\Delta\Psi_m$ is observed, which promotes ROS production [42, 59]. Interestingly, UCP2 also serves as a cellular target of ROS signals such as superoxide, which activates UCP2 to reduce $\Delta\Psi_m$ and lower ROS generation [60]. Recent evidence further supports the notion that rising levels of ROS induce mitochondrial uncoupling through UCP2 activation, a process which also liberates glutathione [27], indicating that under physiological conditions UCP2 is part of an adaptive response to maintain proper redox homeostasis through bidirectional regulation.

Physiological compensation

The physiological role of UCP2 is thus one of compensations to decrease $\Delta\Psi_m$ in the face of cellular stress. UCP2 is central to the diabetes-related stress response of pancreatic islet cells and has been used as a marker of sublethal insult to beta cells [61, 62], eventually resulting in activation of the mPTP [63]. This shift from effective compensation to mPTP activation and decompensation may underlie recent observations that the peroxisome proliferator-activated receptor gamma (PPAR- γ) agonist rosiglitazone, known to cause oxidative damage to cardiomyocytes, has an acute protective effect in cultured cells [14] and neurons [64]. In the case of TBI, the role of UCP2 would be of acute compensation, potentially mitigating damage enough to allow cellular recovery in cases where UCP2 is not overwhelmed. Exemplifying this mechanism is ghrelin, which has been shown to be neuroprotective in the context of TBI through a mechanism reminiscent of ischemic preconditioning, resulting in increased UCP2 expression and greater neuroprotection [7, 8, 65, 66]. In contrast, ghrelin's counterpart leptin has been shown to have more acute UCP2-

dependent mitochondrial stabilization effects in thalamic neurons [64, 67]. It appears that in neurons, UCP2 stabilizes mitochondria by responding to sublethal stress.

Pathophysiology of UCP2

Neurodegeneration

ROS, particularly hydrogen peroxide, have come to be appreciated as important for cellular “redox” signaling to mediate diverse physiological responses [36, 68-70], notably synaptic signaling and plasticity [71], where tight spatial and temporal regulations are likely critical to the ability of ROS moieties to perform physiologic functions with minimal toxicity [72]. The importance of ROS-mediated neurotoxicity in ischemic and traumatic brain injury as well as in chronic neurodegenerative diseases has been well documented, and the presence of free radicals in the brain resulting from mitochondrial dysfunction or excitotoxicity has been shown to contribute to neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and amyotrophic lateral sclerosis [73]. Damage to respiratory chain Complex IV has been shown to contribute to multiple sclerosis pathophysiology by increasing $\Delta\Psi_m$ and augmenting glutamate-mediated axonal injury and associated immune activity and demyelination [74], while robustly functioning mitochondria are associated with faster and more complete recovery [75].

Amyloid- β is recognized as a part of Alzheimer’s disease pathophysiology. While neuroprotective in the initial phase [76], its accumulation is damaging and not unique to Alzheimer’s disease alone: amyloid- β has also been found to accumulate in TBI patients [77-79]. Oxidative stress in the context of TBI was demonstrated to increase production of amyloid in the brain. In controlled studies of TBI, it was found that levels of ROS were elevated within one hour after injury and persisted at elevated levels for more than 4 days [80].

A *post-mortem* human study of neurons from the substantia nigra found that idiopathic Parkinson’s disease demonstrated increased respiratory chain complexes in mitochondria with the same density of

mitochondria [81], possibly representing mitochondrial compensation in response to the suboptimal efficacy of the neuronal system. One recent study identified ursocholic acid as a compound which acted to recover mitochondrial function and normalize ATP levels in fibroblasts from patients with a parkin (*PARK2*) mutation [82]. Importantly, the cell donors were patients with a predisposition to a Parkinson diagnosis, not those with diagnosed Parkinson’s disease. The most attractive feature of such approaches to finding novel treatments of neurodegenerative disease is the recognition that the optimal time to treat is during the subclinical compensatory phase, before failure of the system allows diagnosis.

Reperfusion injury and UCP2

Post-traumatic oxygen delivery is an important part of emergent therapy to prevent complications of TBI, such that hyperbaric oxygen has been studied alongside high-flow nasal cannula to optimize oxygen delivery to brain parenchyma without inducing oxygen toxicity. Both 60-min bursts of hyperbaric oxygen and three-hour treatments with high-flow nasal cannula were shown to be efficacious, increasing cerebral blood flow and decreasing lactate levels with no sign of pulmonary or cerebral damage in patients with severe TBI [83]. Murine hyperoxia has been shown to induce UCP2 within 60 min, perhaps in response to the sudden increase in ROS production, suggesting a mechanism for this protection in which UCP2 was not being transcribed anew but that existing UCP2 mRNA and glutathione-coupled UCP2 were being mobilized; indeed, UCP2 protein level was found to increase with a corresponding decrease in $\Delta\Psi_m$ while the amount of UCP2 mRNA did not change [84]. This result extends specifically to toxicity mediated by hyperactivity associated with seizure in a murine model and is postulated to be at the core of the preconditioning phenomenon of cerebral vascular injury protection [32]. Oxygen delivery is universally recognized as critically important to the preservation of function in cerebral injury, but is also perhaps only important given the assumed preservation of the ability of cerebral parenchyma to utilize the

oxygen with functioning mitochondria [85].

We hypothesize that UCP2 is of universal importance for helping cells overcome periods of stress. UCP2 is also part of the decision-making machinery that pivots the cell from a defensive posture of compensation to an apoptotic state upon decompensation. Through its capacities to regulate cellular energy balance and metabolism, calcium homeostasis, and ROS production, UCP2 plays a critical role in deciding the fate of the cell through mitochondrial membrane permeabilization [4]. For example, the principal trigger for mPTP opening is intramitochondrial calcium in the presence of phosphate – a state consistent with a large $\Delta\Psi_m$ and inefficiently functioning ATP-synthase, which favors ROS production [44, 86]. UCP2 may inhibit the activation of mPTP by mildly depolarizing mitochondria, leading to a decreased uptake of calcium, a reduced production of ROS, and an altered ATP/ADP ratio during compensation [4]. However, this compensation may be overcome at a critical point leading to triggering of mPTP and subsequent cell death. Understanding the underlying mechanism of cellular deterioration may allow new and more effective treatment strategies [87], such as understanding the metabolically distinct state of stressed cells. In this situation, even the biosynthetic pentose phosphate shunt can become clinically toxic [88], as the restoration of normal levels of oxygen and glucose exceeds the limits of this abnormal physiological system and leads to reperfusion injury.

UCP2 induction has been found to be protective against ischemia-reperfusion injury and may be a common downstream factor in multiple pathways including ischemic preconditioning protection in human neurons [64]. In contrast to UCP1, UCP2 does not contribute to core body thermogenesis; however, intrinsic to its ability to uncouple oxidative phosphorylation, UCP2 dissipates energy in the form of heat at the mitochondrial level. Because neurotransmission is highly temperature sensitive [89, 90] and neuronal UCP2-containing mitochondria appear to accumulate in axon terminals [29], experimental data suggest that UCP2 may function to generate a synaptic microenvironmental

temperature gradient and thermally modulate neurotransmission [29, 91]. The temperature-dependent nature of neurotransmitter diffusion and receptor kinetics has been well established [92, 93]. Recent evidence further supports that both synaptic and volume diffusion of neurotransmitters are highly temperature sensitive [89, 90]. Further, neurotransmission is highly sensitive to cellular mechanisms that regulate calcium and ROS homeostasis [50, 71, 94].

Traumatic brain injury (TBI)

The effects of TBI on brain metabolism may also have a central role, and the neuroprotection offered by therapeutic hypothermia has been linked to a reduced blood glucose and lactate level as well as antioxidant preservation [95, 96]; all metabolic effects associated with preservation of mitochondrial functionality. Much more is known about ice-bath induced and cold saline infusion-mediated therapeutic hypothermia, and as the lack of harm associated with pre-hospital cooling has recently been demonstrated [97], more clinical studies are likely to come. Interestingly, a high blood alcohol level at the time of TBI has been associated with improved outcome in therapeutic hypothermia, leading to hypotheses about the optimal composition of intravascular contents during therapeutic hypothermia. One recent study, which demonstrated such a superior outcome for patients that were considered alcohol positive (alcohol ≥ 0.08 g/dL, *i.e.* ≥ 17.4 mmol/L) at the time of admission, opens the possibility that these patients may have been prone to an undeserved 'severe' subjective injury rating due to cognitive effects of inebriation, possibly explaining why severe TBI patients admitted with high blood alcohol levels recovered to a greater extent than their sober counterparts

[98]. We propose that the underlying cellular mechanisms of therapeutic hypothermia be probed in order to identify more clearly how reperfusion injury is mitigated, and we posit that UCP2 may be central both to cellular protection from ROS and to the signaling pathway resulting in decompensation at the cellular level.

Diffuse axonal injury as a consequence of TBI includes direct axonal injury and myelin disruption and leads to major impairment of many functional pathways [99]. With the disruption of the integration necessary for cognitive and executive function, patients may be significantly impaired, though new rehabilitative techniques are emerging that can help to improve higher-order functioning at least in mild TBI cases [100]. Gaining an understanding of the cellular processes which govern the response to and control of damage, such as those involved in apoptotic signaling or reducing ROS exposure, would offer a method of reducing axonal injury or enhancing recovery in the early post-traumatic phase.

Hypothermia seeks to decrease ROS production by decreasing energy demand, but has significant issues in terms of methods applied to achieve cooling [101, 102]. With a greater understanding of the metabolic pathways responsible for the reduced ROS-associated cellular damage as a result of cooling, one could pharmacologically target those pathways and allow rapid and effective pre-hospital or in-hospital cooling effects without dependence on ambulance travel times or comorbidities such as chronic heart failure that may preclude some cooling strategies [101]. Given that UCP2 would dissipate $\Delta\Psi_m$ through heat generation and release, some manner of cooling would likely remain necessary but it is possible that the target temperatures may be significantly closer to normal body temperature given the targeted nature of such

a therapy. The key to the attractiveness of a UCP2 activation approach to cooling is that as excess $\Delta\Psi_m$ dissipates and the danger of ROS-mediated damage is reduced, the substrate for UCP2-generated heat would also be reduced in real time, offering not only a tunable property in $\Delta\Psi_m$ response to such a therapy but also a prognostic indicator in the form of clinically measureable temperature changes.

Conclusion

Early intervention to prevent initial neuronal injury extension after TBI is critical for TBI recovery, and UCP2 may offer an important target of early treatment. The efficient delivery of oxygen and glucose to neural tissues after injury has been emphasized [83, 103], but the usefulness of these depends upon the retention of cellular ability to process and utilize them [85]. Post-injury mitochondrial uncoupling can increase tissue salvaged and cognition retained in model systems [104] and may be at least as important as maintaining oxygenation in ischemic neural tissue [85]. While the pleiotropic effects of hypothermia have been shown to be efficacious [105] and may include the preservation of mitochondrial function [106], the targeting of UCP2 may provide a specific and reliable treatment for TBI patients to minimize injury and maximize recovery. New techniques are emerging at both the subcellular level [53] and the tissue level [107] to understand the processes underlying TBI, and the development of new techniques to more specifically prevent damage to brain parenchyma will undoubtedly result in improved patient outcomes.

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Conflict of interest statement: The authors declare that there are no conflicts of interest.

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