Insight into a neuron’s preferential susceptibility to oxidative stress

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Abstract

Neurons are more vulnerable to oxidative stress than astrocytes, the reasons for which have yet to be fully elucidated. Understanding the cellular and molecular mechanisms which contribute to this enhanced vulnerability is key to efforts aimed at ameliorating neuronal health and resilience to oxidative stress, particularly in the context of neurodegenerative disease, which is characterized by progressive dysfunction and loss of neurons specifically, and in which oxidative stress is considered a central aetiologial contributor. Biological factors which may influence neuronal susceptibility to oxidative stress, in normal and neurodegenerative contexts, are reviewed in the present article, with a focus on properties intrinsic to the neuronal cell type and on properties related to neuronal reliance on surrounding astrocytes.

Introduction

According to the theory of neuronal health, neurons exist in a dynamic spectrum of states, which range from highly resilient and fully functional to vulnerable and dysfunctional. External cues can beneficially or detrimentally influence a neuron’s position within this spectrum, affecting the resultant ability to sustain insult [1]. One factor with a known capacity to weaken neuronal health is OS (oxidative stress), which arises from an imbalance between oxidant-producing systems and intrinsic antioxidant defences, ultimately resulting in an accumulation of ROS (reactive oxygen species). ROS are oxygen-containing intermediates with the capacity to oxidize surrounding biomolecules. At low levels, ROS participate in key redox-dependent signalling pathways, but at high levels, ROS accumulation can cause detrimental oxidative modifications of lipids, proteins and nucleic acids, the hallmarks of OS. Thus increased ROS production or decreased ROS scavenging can profoundly influence redox status and neuronal health. To maintain ROS within biologically safe thresholds, cells are armed with intrinsic antioxidant defences, which neutralize unwanted ROS and maintain redox homeostasis.

Although OS occurs throughout the body, the brain is an especially vulnerable organ because of its high requirements for oxygen consumption, its abundance of redox-active metals and its high concentration of oxidizable polyunsaturated fatty acids. Interestingly, although the brain as a whole is highly vulnerable to OS, the subcellular resistance to OS is heterogeneous, with neurons being a particularly vulnerable brain cell type, as is evident by the

Key words: antioxidant defence, astrocyte, neurodegeneration, neuron, neuroprotection, oxidative stress.

Abbreviations used: Aβ, amyloid β-peptide; AD, Alzheimer’s disease; HD, Huntington’s disease; HNE, 4-hydroxy-2-nonenal; MND, motor neuron disease; NMDA, N-methyl-D-aspartate; 8-OHG, 8-hydroxyguanosine; OS, oxidative stress; PD, Parkinson’s disease; Prx, peroxiredoxin; RNS, reactive nitrogen species; ROS, reactive oxygen species.

Introduction

ROS: friend and foe

ROS are oxygen-carrying metabolites which may or may not contain a free radical, but which share the ability to oxidize other cellular components. Although most often seen as a dangerous event, ROS production is an unavoidable and biologically important occurrence within cells. Cellular respiration accounts for the vast majority of biologically produced ROS, as the incomplete reduction of molecular oxygen leads to the generation of superoxide radicals (O2•−) within the mitochondrial electron transport chain. NADPH oxidase, xanthine oxidase and monoamine oxidase are also sources of ROS produced by means of physiologically required processes. Superoxide radicals can undergo spontaneous dismutation to form hydrogen peroxide (H2O2), a lipid-permeant ROS, which readily diffuses through cell membranes. Peroxide’s oxidizing activity can cause haem protein degradation, enzyme inactivation and oxidation of lipids, proteins and thiol-containing molecules. Peroxide’s most potentially dangerous interaction, however, is with surrounding metal ions, as this can trigger the formation of Fenton-based hydroxyl radicals (OH•), a highly reactive ROS, which readily attacks most cellular components. Given the high abundance of metal ions in the brain, OH• formation from H2O2 is a particularly relevant concern for neural cells. Superoxide radicals can also give rise to more dangerous ROS including alkoxyl radicals (RO•), peroxyradicals (ROO•) and hypchlorous acid (HOCl), as well as to peroxynitrite (ONOO−), an RNS (reactive nitrogen species) arising from...
the reaction of superoxide radicals with nitric oxide (NO\(^*\)). Peroxynitrite can damage a wide array of biomolecules, including proteins and DNA, and can also give rise to nitrogen dioxide (\(\text{NO}_2\)) and dinitrogen trioxide (\(\text{N}_2\text{O}_3\)). See Figure 1 for a diagrammatic representation of ROS/RNS.

Under normal physiological conditions, ROS levels are low and ROS detoxification is easily managed by intrinsic antioxidant defence systems. In this context, ROS benefit neuronal health because of their central role in redox signalling. Redox-dependent signalling pathways are made up of redox-sensitive proteins that can undergo reversible oxidation or reduction reactions. The function of redox-sensitive proteins change depending on whether a protein is in its oxidized or reduced state. Thus ROS–RNS interaction influences protein signalling capacity in a redox-dependent manner. ROS and RNS play a key role in a variety of biologically important neural pathways including LTP (long-term potentiation), synaptic plasticity, neurogenesis, neuronal differentiation, apoptosis and the establishment of neuroprotective preconditioning [3,4]. Cellular signalling is also influenced by redox status at the transcriptional level, as numerous transcription factors are redox-sensitive, including NF-κB (nuclear factor κB), HIF (hypoxia-inducible factor), AP-1 (activator protein 1) and Nrf2 (nuclear factor-erythroid 2-related factor 2) [5].

If improperly neutralized or eliminated, ROS/RNS transition from friend to foe as increased accumulation disrupts cellular redox pathways and enables unregulated interaction with surrounding biomolecules. To keep detoxification in line with production, the cell employs a host of antioxidant enzymes and non-protein antioxidants. Given that superoxide anions are the most abundant biologically produced ROS with a capacity to trigger further ROS/RNS formation, their neutralization is key to maintaining cellular redox status and neuronal health. Superoxide anions can be converted into peroxide by the activities of SOD (superoxide dismutase) and catalase, which can then be converted into water by members of the glutathione and Prx (peroxiredoxins)–Trx (thioredoxins) families [GPx (glutathione peroxidase) and Prx respectively]. Non-protein antioxidants include glutathione and bilirubin (a member of the haem degradation pathway), which directly quench hydroxyl radicals. Exogenous antioxidants, which are sourced from the diet, act in concert with endogenous antioxidants, and include α-tocopherol, ascorbic acid and carotenoids.

Mild OS is a normal response to biologically produced ROS, and is generally short-lived due to rapid ROS/RNS elimination. OS is detrimental to neuronal health when its magnitude exceeds the elimination capacity of antioxidant defences. Unregulated ROS/RNS interaction with surrounding biomolecules can cause detrimental events such as oxidative or nitrosative modifications of proteins and nucleic acids, lipid peroxidation and carbonyl formation. In an effort to prevent cellular dysfunction, defensive mechanisms are employed to rectify OS-induced damage including protein refolding or degradation, lipid turnover and DNA base excision and repair.

In the context of disease, the impact of OS on neuronal health is exacerbated by pathology-dependent ROS/RNS production. Whereas AD, PD, HD and MND arise from distinct neuropathologies, they are linked by evidence supporting OS as a central component of disease aetiology. Indeed, post-mortem PD brain tissue shows an increase in protein carbonyls, 4-HNE (4-hydroxynonenal), 8-OHG (8-hydroxyguanosine) and nitrotyrosine, markers of protein oxidation, lipid peroxidation, DNA oxidation and protein nitrosylation respectively [6–8]. Evidence of OS also exists within the AD pathology, where abnormalities in mitochondria and mtDNA, RNA oxidation, carboxyl-related post-translational modifications, nitrosative modifications and an up-regulation of antioxidant defences have been observed [9–13]. In the HD pathology, markers of oxidative damage are also increased in affected brain areas, including 3-nitrotyrosine (a marker of peroxynitrite-mediated protein nitration), lipofuscin (a product of unsaturated fatty acid peroxidation), 8-OHG and 4-HNE [14]. Finally, a similar presence of OS is visible in the spinal cord or cerebrospinal fluid of subjects diagnosed with MND, as markers of oxidative damage including 8-OHG, protein tyrosine nitration, protein carbonyl levels and NO\(^*\) products are all increased [15].

## Neurons exhibit increased vulnerability to oxidative stress

AD, PD, HD and MND (as well as other neurodegenerative diseases) are unified by evidence of OS-mediated damage, and by a progressive dysfunction and loss of neurons; in AD by the loss of synaptic contacts and neurons in cortical brain regions, in PD by the progressive degeneration of nigrostriatal neurons, in HD by a loss of striatal

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**Figure 1** | Diagrammatic representation of the generation of ROS and RNS (in red) from molecular oxygen

Key ROS and RNS members are shown in red: superoxide (O\(_2\)\(^{•−}\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radicals (OH\(^{•}\)), alkoxyl radicals (RO\(^{•}\)), peroxy radicals (ROO\(^{•}\)), hypochlorous acid (HOCl), nitric oxide (NO\(^{•}\)), peroxynitrite (ONOO\(^{−}\)), nitrogen dioxide (\(\text{NO}_2\)) and dinitrogen trioxide (\(\text{N}_2\text{O}_3\)).
medium-sized spiny neurons, and in MND by the death of upper and lower motor neurons. In contrast with neuronal depletion, astrocyte number is actually increased in AD, PD, HD and MND, and, most importantly, this increase in number is associated with a maintenance of functional capacity; astrocytes localize intimately with pathological lesions, display an up-regulation of activity and inflammatory markers and show internalization of pathological protein inclusions [7,16,17]. In AD brain tissue, protein oxidation, lipid peroxidation, peroxynitrite-induced damage and RNA depletion were all found to occur specifically in neurons, not astrocytes [7,9,13,17,18].

In addition to evidence of an enhanced susceptibility of neurons to OS in humans, increased neuronal vulnerability to OS has also been extensively observed experimentally. Oxidative insults have been shown to cause greater ROS production, greater glutathione depletion, greater loss of mitochondrial membrane potential, greater loss of intracellular ATP concentration, lower induction of antioxidant enzymes and higher rates of cell death in neurons, compared with astrocytes (see, e.g., [19–22]).

**Possible contributing factors to neuronal susceptibility to oxidative stress**

Although excess production of ROS/RNS causes OS in all cell types, neurons appear to be particularly weak in their ability to recover from oxidative insults and OS-induced damage. This circumstance is most likely to originate from properties intrinsic to the neuronal cell type, as well as from the dependence of neurons on surrounding astrocytes for metabolic and antioxidant support.

**Intrinsic factors contributing to neuronal vulnerability to oxidative insult**

Astrocytes localize to fairly small non-overlapping astrocytic domains. In contrast, neurons can have extremely long-reaching tortuous projections, with complex dendritic arborizations and axonal branching patterns. The metabolic demands associated with such structural complexity, as well as with action potential propagation, synaptic transmission, long-distance molecular trafficking and maintenance of resting membrane potential, mean that neurons have a far greater energetic demand than other cell types. In fact, neurons display a significantly higher intracellular oxidative state than astrocytes [19], which probably contributes to their decreased ability to withstand oxidative insult.

Mitochondria are responsible for the energy production required to meet cellular metabolic demand. For neurons, where energy demands are especially high, this is an especially perilous circumstance given that mitochondria produce both ATP and ROS, often placing them as OS initiators. One of the mechanisms mitochondria utilize to balance intra-organellar ROS production is the import of cytosolic glutathione through glutathione transporters. Interestingly, astrocytes were recently identified as expressing two distinct glutathione transporters, in contrast with neurons, which were only found to express one, a discrepancy which had a direct impact on intramitochondrial glutathione levels and resultant resistance to oxidative and nitrosative insults [24]. ROS quenching within the mitochondria is also critical as mtDNA generally sustains 2–3-fold greater oxidative damage than nuclear DNA because of its close proximity to ROS generation [25]. This is particularly important given the observation that mtDNA repair enzymes are specifically compromised in neurons, compared with repair-proficient astrocytes [19] and that mutations in mtDNA generally impair ATP production and perturb oxidative phosphorylation cascades, compromising cellular viability. Non-mitochondria-based ROS production may also contribute to enhanced neuronal cell death, as excitotoxicity, which results from the uncontrolled activation of NMDA (N-methyl-D-aspartate) receptors is a superoxide-dependent process [26,27], originating from Ca²⁺-dependent activation of cytosolic NADPH oxidase [28] and xanthine oxidase [29]. If un-neutralized, NADPH-sourced superoxide would exacerbate pre-existing ROS, and further weaken neuronal health and resilience. Finally, the fact that neurons are post-mitotic cells with a limited capacity for regeneration means that, unlike astrocytes, the accumulation of OS-induced damage last the lifetime of the cell, greatly increasing its impact on cell health.

**Neurons rely heavily on surrounding astrocytes**

Neurons depend on surrounding astrocytes for a multitude of biologically crucial events including pH and K⁺ buffering, lactate shuttling and glycogen storage, and neurovascular coupling, which for the purpose of the present review, will not be discussed further (for further details, see [30,31]). Astrocytes also act as a physical barrier for neurons, as they surround cell bodies, neuronal processes and synapses, shielding neurons from exposure to ROS/RNS or glutamate. Astrocytes control further a neuron’s exposure to neurotransmitters such as glutamate via modulation of transmitter release and absorption. This ability is particularly important to neuronal health as impaired glutamate signalling (due to excessive transmitter release or possible reversal of glutamate uptake transporters) increases ROS formation (via the aforementioned activation of NADPH and xanthine oxidases) and can lead to detrimental activation of extrasynaptic NMDA receptors, triggering pro-death signalling cascades [32].

What is perhaps the most relevant to a neuron’s enhanced sensitivity to OS, however, is its dependence on astrocytes for antioxidant support, particularly with regard to glutathione. Astrocytes have higher levels of antioxidant enzymes, including glutathione and catalase, and higher rates of glutathione synthesis, compared with neurons [19,33–38]. Neurons routinely fare better if co-cultured with astrocytes [33,39,40], because of astrocytic glutathione release [41,42], which can scavenge free radicals in the extracellular space [43] and provide cysteine, a transportable glutathione precursor, which enables neuronal glutathione formation [41,42]. Neuronal health can also be boosted by the provision
of ascorbic acid from astrocytes, which can quench free radicals directly or be used as a reducing equivalent in glutathione recycling.

Pathology exacerbates pre-existing neuronal vulnerability to oxidative stress

Neurons are placed in a tenuous position by their dependence on astrocytes for metabolic and antioxidant support. A neuron’s inability to be self-sufficient renders it vulnerable to insults occurring in both neurons and astrocytes, as a compromise in astrocyte function would be reflected in astrocytic capacity for neuronal support. Despite this, it is clear that, under normal physiological circumstances, neuronal antioxidant capacity is sufficient to maintain neuronal function and resistance to the small levels of OS associated with biological ROS production. However, the added burden of neuropathology or insult probably represents a tipping point where oxidative burden outweighs neuron and astrocyte-sourced antioxidant defences specifically in neurons, but not in astrocytes. In this regard, it is interesting to note that the biggest risk factor for neurodegenerative disease is age, which, according to the OS theory of aging, is itself a result of oxidative damage accumulation. Thus, even before the exposure of disease-associated pathology, neuronal health in aged neurons is likely to be already compromised by aging-associated OS.

In addition to aging, there are other sources of OS, common to AD, PD, HD and MND, which also contribute to neuronal demise. Because of its pivotal role in both ATP synthesis and ROS generation, impaired mitochondrial function is central to OS-induced neurodegeneration, and is evident pathologically by respiratory chain dysfunction, decreased ATP production, Ca\(^{2+}\) dysregulation and alterations in mitochondrial morphology and clearance [7,44]. The presence of activated microglia and astrocytes, a hallmark feature of neurodegeneration, also exacerbates OS, given the utilization of NO\(^*\) and ROS to scavenge protein deposits [17]. Neurodegeneration is also linked with accumulation of advanced glycation end products and redox metals, as well as by an elevation in nitrosylated protein-disulfide isomerase (a protein with a key role in the maintenance of endoplasmic reticulum redox status and protein misfolding) [2,7,44,45], which would enhance ROS production and worsen OS.

In addition to these generalized features of neurodegeneration, OS can also arise from pathological changes unique to a specific disease type. For example in AD models, A\(_{β}\) (amyloid \(β\)-peptide) and tau proteins have been linked to ROS production following association with metal ions [9,13]. This is particularly problematic given that OS itself increases the expression of enzymes involved in A\(_{β}\) production, thereby creating a detrimental cycle of OS-induced events [13]. Dopaminergic neuronal demise in PD is associated with increased ROS production in the substantia nigra, due partly to an increase in iron content, but also to the metabolism or auto-oxidation of dopamine itself, as this produces peroxide and superoxide respectively [6,8]. Mitochondrial function in HD is exacerbated further by decreased expression of PGC-1\(α\) (peroxisome-proliferator-activated receptor \(γ\) co-activator 1\(α\)), a master transcriptional co-regulator of mitochondrial biogenesis and antioxidant enzymes, which impairs mitochondria and worsens ongoing OS [14].

Conclusions

The hypothesis advanced in the present review is that neurons are more vulnerable to OS because of both intrinsic properties and their reliance on surrounding astrocytes for a multitude of biologically important events, including antioxidant support. The reviewed studies underscore the profound influence astrocytes have on neuronal health under both normal and pathological contexts, and suggest that the classically neurocentric view of neurodegeneration should be expanded to include an assessment of astrocitc health and function, as neuronal demise would undoubtedly be accelerated by astrocytic decline. Indeed, an enhancement of antioxidant defences, specifically in astrocytes, has been shown to improve neuronal health and viability in a variety of experimental models of neurodegeneration (for a review, see [46]).

References
