Reactive Oxygen Species in Neurons

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Reactive oxygen species (ROS) have emerged as regulators of key processes supporting neuronal growth, function, and plasticity across lifespan. At normal physiological levels, ROS perform important roles as secondary messengers in diverse molecular processes such as regulating neuronal differentiation, polarization, synapse maturation, and neurotransmission. In contrast, high levels of ROS are toxic and can ultimately lead to cell death. Excitable cells, such as neurons, often require high levels of metabolic activity to perform their functions. As a consequence, these cells are more likely to produce high levels of ROS, potentially enhancing their susceptibility to oxidative damage. In addition, because neurons are generally post-mitotic, they may be subject to accumulating oxidative damage. Thus, maintaining tight control over ROS concentration in the nervous system is essential for proper neuronal development and function.

synapse oxidative stress neurodevelopment neurotransmission

1. Introduction

Oxygen-derived free radicals, such as the superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$), as well as non-radical molecules, such as hydrogen peroxide (H_2O_2), are collectively known as reactive oxygen species (ROS) [1][2]. ROS are produced as metabolic by-products in different subcellular locations [1][3], including mitochondria [4], endoplasmic reticulum [5], peroxisome [6], plasma membrane [7] and cytosol [8]. Exposure to pathogens, harmful chemicals, heat, UV radiation [9], and heavy metals [10] that potentially damage mitochondria can induce cycles of ROS production and increase cellular ROS concentration. ROS are highly reactive and have the capacity to interact with different biomolecules. At high concentration, ROS can damage DNA, protein, and lipid and disrupt the plasma membrane [1][11], ultimately leading to cell death [12]. Oxidative damage is implicated in several pathological conditions, including major neurodegenerative diseases such as Alzheimer's and Parkinson's disease [13], muscular dystrophy [14], and other disease conditions such as chronic inflammation and tissue injury [15], diabetes mellitus [16], and cancer [17]. Further, toxic oxidative metabolites can potentiate necrosis and apoptosis following neuronal injury [18]. To protect from the deleterious effects of oxidative damage, healthy cells need to be equipped with dedicated protective mechanisms such as antioxidant synthesis. Antioxidants can be either enzymatic (superoxide dismutase, catalase, glutathione peroxidases) or nonenzymatic biochemicals (flavonoid, ascorbic acid, tocopherol, etc.) [19]. When antioxidants fail to maintain the cellular ROS concentration within an appropriate physiological range, oxidative stress occurs.

2. ROS in Neurodevelopment

ROS signaling is an important regulator of neuronal differentiation and polarization, axon outgrowth, synapse formation, and synapse maturation [3][20].

2.1. ROS in Neurogenesis and Differentiation

Neurons are generated from neural stem cells and neuronal progenitors during neurogenesis. NADPH oxidases, particularly NOX2, are expressed in the developing brain during embryogenesis [20][21]. Cortical progenitors and progenitor-derived neurons actively synthesize ROS [22], suggesting that ROS may perform signaling roles in early neurogenesis. Consistent with this idea, ROS levels influence the timing of neurogenesis in the murine embryo [23]. Neuronal differentiation from primary neuronal progenitor cells and the establishment of neuronal identity depend on a complex array of biochemical interactions between growth factors such as nerve growth factor (NGF) [24] and other molecular regulators. NGF treatment triggers an increase in ROS in cultured PC12 cells. Prevention of ROS production inhibits NGF-triggered differentiation of PC12 cells, indicating that ROS may have important intracellular signaling roles during neuronal differentiation [25]. Additional evidence for ROS regulation of neuronal differentiation comes from studies of Neuregulins, a large family of EGF-like signaling molecules that are highly expressed in the nervous system. Neuregulin activation of ErbB receptor tyrosine kinases induces neurite outgrowth in PC12 cells through activation of the MAP kinase signaling pathway [26]. Interestingly, neuregulin treatment was also found to increase intracellular ROS. Treatment with a ROS scavenger inhibited both neuregulin-induced Ras and ERK activation and neuronal differentiation, implicating ROS in the molecular regulation of neuregulin-mediated differentiation [27]. Further investigation revealed that the kinetics of cellular ROS production is an important factor in the cellular decision to divide or differentiate [27].

Recent studies also provide evidence that ROS can act as signals for adult neurogenesis in both the central [28] and peripheral nervous systems [29]. Endogenous H_2O_2 regulates growth signaling to maintain proliferating adult hippocampal progenitor cells [30]. Similarly, inhibition of ROS biosynthesis retards proliferation and neurogenesis of neural stem cells in the adult newt brain [31]. These reports and additional accumulating evidence argue for a reconsideration of the view that ROS serve primarily deleterious roles in the nervous system and support a new acknowledgment of roles for ROS signaling in both embryonic and adult neurogenesis and differentiation.

2.2. ROS in Neurite Outgrowth and Polarization

The outgrowth of polarized axons and dendrites is a critical step in the development of brain circuits. Interestingly, ROS have emerged as positive regulators of this process. NOX synthesis of H_2O_2 can induce axon and dendrite formation and maturation [20]. Inhibition of the NOX complex disrupts the timing of neuronal polarization, shortens axonal length, and alters the actin cytoskeleton of cultured hippocampal neurons [32]. Furthermore, ROS regulate actin cytoskeletal dynamics and control neurite outgrowth in Aplysia neurons [33]. Recent evidence also suggests a potential role for ROS in the process of axonal specification. The polarity of hippocampal neurons is defined by a polarized distribution of evolutionarily conserved polarity proteins, such as mPar3 and mPar6. Phosphatidylinositol 3-kinase pathway (PI3′K) signaling is critical for achieving this polarized distribution [34]. Physiological levels of ROS have been shown to influence PI3′K signaling by inactivating phosphatase and tensin homolog (PTEN) [35]. It

will therefore be interesting to investigate how changes in neuronal redox state might impact the subcellular distribution of polarity proteins and the selection of future axons. Normal physiological levels of ROS support neurite outgrowth and potentially axonal specification, but what molecular mechanisms are instrumental for maintaining physiological ROS concentrations in neurons? Recent findings have offered evidence that a novel feedforward mechanism involving NOX-mediated ROS production and intracellular Ca^{2+} signaling may have a central role [36]. Ryanodine receptor (RyR)-mediated Ca^{2+} release from the endoplasmic reticulum promotes axon extension and is supported by NOX-generated ROS. RyR activation also induces H_2O_2 production by NOX through a Rac-1 (Rac Family Small GTPase 1) dependent mechanism [36], offering a potential route for feedforward regulation.

2.3. ROS Influence Growth Cone Guidance and Synaptic Maturation

Critical steps in the development of the nervous system include the growth of axons to meet their cellular partners and the subsequent formation of specialized connections called synapses. Axon outgrowth is a complex process involving dynamic changes in the neuronal cytoskeleton. These cytoskeletal dynamics are important for guiding the growing tip of the axon (growth cone) to its destination and are regulated by attractive or repulsive cues in the extracellular environment. Semaphorin is a major repulsive cue for axons, and semaphorin signaling can induce the collapse of growth cones [37]. Semaphorin3A (Sema3A) triggers microtubule disassembly through phosphorylation of the cytoskeletal component regulator, collapsin response mediator protein 2 (CRMP2) [38]. Sema3A triggers an increase in H_2O_2 in the growth cones of dorsal root ganglion axons through activation of the multidomain redox enzyme Mical. Subsequently, H_2O_2 oxidizes CRMP2, enabling phosphorylation by glycogen synthase kinase-3 (GSK-3) and promoting growth cone collapse [39]. Another recent study showed that NOX2 acts downstream of the Slit2/Robo2 signaling pathway during the growth and guidance of retinal ganglionic cell (RGC) axons in the zebrafish embryo [40]. Related studies have implicated ROS in the regulation of the growth of synapses. A genetic screen for mutants with synaptic overgrowth at the larval neuromuscular junction of *Drosophila* identified *spinster* mutants [41]. The causal mutation in *spinster* is a loss of function mutation in a putative lysosomal efflux permease. Lysosomal dysfunction in *spinster* mutants leads to an increased ROS burden. Interestingly, reduction of ROS in *spinster* mutants normalized the synaptic overgrowth phenotype, suggesting a link between synaptic overgrowth and oxidative stress [42]. Further work implicated ROS activation of the JNK pathway in synaptic growth [42].

Taken together, the findings described in these studies support an expanded view of physiological ROS regulation of key neurodevelopmental events, including axon guidance, synapse formation, and synapse maturation.

2.4. Mitochondrial ROS Facilitate Synaptic Pruning by Intrinsic Apoptosis

In most nervous systems, there is an excess of synaptic connections during early development compared with at maturity. Juvenile connections often undergo a refinement process called synaptic pruning, in which weaker or inappropriate connections are eliminated to generate the mature circuit [43][44]. Significant questions remain about the molecular pathways that identify such synapses and initiate their elimination. Mitochondrial ROS are emerging

as potential cell-intrinsic factors important for synapse elimination [45][46]. Recent studies of motor behavior in *Xenopus* tadpoles suggest a regulatory role for mitochondrial ROS in synaptic pruning at the neuromuscular junction (NMJ) [46]. The researchers found that forced synaptic inactivity increased mitochondrial ROS and mitochondria-targeted antioxidants reduced motor deficits associated with endogenous pruning. Interestingly, this model challenges the prevalent idea that increased neuronal activity is linked with an increase in mitochondrial ROS generation [47][48]. A follow-up communication from this group provided a hypothetical model accounting for their findings, which suggests that neuronal activity may mask a cue for pruning by suppressing mitochondrial ROS production [45]. Mitochondrial O_2^-/H_2O_2 concentration would therefore surpass the pruning threshold only at inactive synapses, thereby locally activating intrinsic apoptotic cell death signaling pathways to initiate synapse elimination or pruning [45]. Importantly, intrinsic apoptosis can be initiated, propagated and amplified by mitochondrial ROS [49][50][51]. Related studies of *C. elegans* neurons have shown that elimination of presynaptic material involves axonal mitochondria and apoptotic signaling [52], though specific roles for ROS in this process were not determined. An enhanced understanding of the molecular mechanisms that relate neuronal activity to ROS generation and their influence on the stability of synapses will perhaps emerge from ongoing investigations in this area.

2.5. Oxidative Damage in Neurodevelopmental Diseases

Interestingly, while physiological ROS have emerged as positive regulators of the processes underlying neuronal development, mounting evidence has also linked oxidative stress and damage with the pathophysiology of neurodevelopmental and neuropsychiatric diseases. Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental abnormalities that manifest as social and cognitive impairments in children and young adults. Post-mortem brain samples from temporal cortices and cerebella of autistic subjects showed a decrease in antioxidants such as glutathione (GSH) and reduced GSH/GSSG redox/antioxidant capacity [53]. Additionally, red blood cells of autistic children have altered antioxidant (SOD, catalase, and GSH) levels compared to controls [54][55]. For a detailed description of oxidative stress in ASD, readers should refer to the review by Pangrazzi [56]. Oxidative stress is also thought to influence the progression of disease pathology in schizophrenia patients [57][58]. Proteomic and metabolomic analyses of superior temporal gyrus and prefrontal cortex tissues from schizophrenic individuals revealed mitochondrial dysregulation, compromised brain metabolism, and oxidative stress [59][60]. Intriguingly, low levels of antioxidant enzymes such as GSH have also been reported in blood samples from patients with schizophrenia [57][61][62]. Because enzymatic activity remained unchanged [63], this result is most consistent with a reduction in GSH synthesis by antioxidant producing cells. Malondialdehyde (MDA) is generated by the peroxidation of membrane polyunsaturated fatty acids and is commonly used as a biomarker to assess oxidative stress [64]. High MDA levels have been reported in adult attention-deficit hyperactivity disorder (A-ADHD), which may point toward an association with oxidative stress [65][66][67]. Consistent with this, increased levels of MDA and decreased levels of the MDA catabolic enzymes paraoxonase and arylesterase were reported in patient serum. However, no correlation between these molecular parameters and disease severity was found [67]. Most studies investigating oxidative stress in the context of neurodevelopmental disease are heavily reliant on analysis of post-mortem tissue or blood serum. It is therefore difficult to assess causality from these analyses. Is oxidative damage a result of, or a cause of, neurodevelopmental abnormalities? Further investigation into important

questions surrounding how ROS can both positively and negatively affect neurodevelopment will be critical for teasing apart this central problem of cause and effect. Future exploration of the molecular basis of oxidative damage in neurodevelopmental diseases may also help to identify novel drug targets and potential therapies.

3. Roles for ROS in Mature Neurons

3.1. ROS in Synaptic Plasticity

There is mounting evidence that reactive oxygen species contribute toward regulation of core neuronal functions such as neurotransmission and synaptic plasticity. Synaptic plasticity is the cellular foundation for learning and memory and refers to structural and molecular modifications at synapses that influence the strength of communication between neurons [68]. The cross-talk between ROS, Ca^{2+} influx, and age-related deficits in the synaptic plasticity of hippocampal neurons has been extensively reviewed elsewhere [69]. Likewise, there is a wide literature available exploring the link between ROS, synaptic plasticity, and memory [70][71][72]. In *Drosophila* larvae, neuronal ROS were found to be instrumental for neuronal activity dependent structural plasticity of both pre and postsynaptic terminals [48]. Moreover, embryonic and larval motor neurons of *Drosophila* use ROS as key messengers in dendritic plasticity. For example, a recent *Drosophila* study showed that neuronal activity triggered extracellular H_2O_2 synthesis by Dual Oxidase. ROS entry into the neuron required neuronal aquaporin expression and was required for structural changes in dendritic arbors [73]. Recent work from *C. elegans* showed that intracellular ROS can modulate the transport and synaptic localization of AMPA-type glutamate receptors through regulation of neuronal Ca^{2+} signaling [74]. Further, studies in hippocampal neurons showed that strong Ca^{2+} transients prolong the lifetime of phosphorylated CREB, a key molecule involved in long-term memory, through enhanced mitochondrial super oxide production [75]. Changes in neuronal oxidative state may also alter Ca^{2+} signaling events that influence hippocampal memory formation [70]. Collectively, the available literature suggests that ROS regulate synaptic plasticity and memory formation in a Ca^{2+} dependent manner.

3.2. ROS Influence Neurotransmission

Redox influences on neurotransmission have been described for small-molecule neurotransmitters. A pioneering study at the lobster neuromuscular junction showed that H_2O_2 exposure decreased release of the excitatory neurotransmitter glutamate [76]. There are numerous reports of ROS effects on GABA-mediated inhibitory neurotransmission and these have been extensively reviewed [77]. In particular, patch clamp recordings from cultured mouse hippocampal neurons and CA1 pyramidal neurons in hippocampal slices showed H_2O_2 directly modulates $GABA_A$ receptor function [78]. Studies of the frog neuromuscular junction showed that exposure to Zn^{2+} and Cd^{2+} enhances mitochondrial ROS levels, leading to desynchronization of cholinergic neurotransmitter release [79]. This effect was ameliorated by antioxidant treatment. More recent *C. elegans* studies have provided evidence that axonal mitochondria and ROS production can also modulate neuropeptide release. Blocking mitochondrial transport into axons or disrupting oxidative phosphorylation inhibited neuropeptide release [80]. In addition, increases in endogenously produced H_2O_2 from axonal mitochondria were shown to enhance neuropeptide secretion [81]. Changes in diet were sufficient to trigger rapid alterations in endogenous ROS. Elevated

neuropeptide secretion in response to endogenous ROS triggered transcriptional activation of antioxidant response genes, offering a potential mechanism for neural control of oxidative stress responses. These studies point toward a role for ROS as secondary messengers that promote neuropeptide secretion.

While there is an increasing appreciation that ROS-mediated signaling plays a positive role in the regulation of neuronal development and function, abundant evidence also links ROS dysregulation with aging neurons and neurodegenerative disease.

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