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FORUM EDITORIAL

Regulation of Neuronal Oxidative and Nitrosative Stress by Endogenous Protective Pathways and Disease Processes

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Abstract

Oxidative/nitrosative stress contributes to the etiology of many neurological disorders in the developing and aged/mature central nervous system, including acute trauma such as ischemia and hyperoxia, as well as chronic diseases such as Alzheimer's and Parkinson's diseases. In addition to the accumulation of nonspecific oxidative damage, it is becoming clear that pathological conditions lead to the oxidative/nitrosative modification of specific proteins, including those involved in apoptosis, proteolysis, and protein (mis)folding. Several disorders, including stroke and Parkinson's disease, are associated with inactivating modifications of antioxidant enzymes themselves, thus compromising antioxidant defenses. Conversely, neuroprotective pathways, such as neurotrophin- and synaptic activity-induced signals, can upregulate key antioxidant systems, potentially contributing to the cytoprotective actions of adaptive stress responses following exercise or calorie-restriction. On the flipside, hypofunction of these pathways is associated with the death of developing neurons. An increased knowledge of how neuronal antioxidant systems are controlled in health and disease is unearthing therapeutic targets in several disorders. Moreover, the emerging importance of master regulators of antioxidant defenses such as nuclear factor-erythroid 2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) is revealing ways through which intrinsic defenses may be manipulated to combat oxidative/nitrosative stress. Such approaches offer an alternative strategy to classical antioxidant interventions based on the administration of free radical scavengers and spin-traps. *Antioxid. Redox Signal.* 14, 1421–1424.

Antioxidant Defenses in the Nervous System

Correct redox control is essential in all cells, particularly in postmitotic cells such as neurons where oxidative damage can accumulate. Oxidative/nitrosative damage and stress occurs due to an imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and the cell's ability to neutralize them through its intrinsic antioxidant defenses. Neurons are highly susceptible to oxidative damage due to high levels of ROS/RNS production (*via* respiration and metabolism) and relatively low amounts of certain antioxidant enzymes, such as catalase (8, 17). Vulnerability of the central nervous system (CNS) to oxidative and nitrosative stress appears to be particularly acute at both ends of life. The developing brain has a high rate of oxygen usage, which, coupled with low levels of antioxidants and high levels of heavy metal ions, can lead to susceptibility to elevated ROS levels. Posttranslational modifications of proteins by RNS often predisposes them to further reaction with less ROS, such as superoxide anion (7). Oxidative stress is associated with neuronal injury and death in the infant brain when subjected to many types of insult, including hyperoxia, ischemia, and traumatic brain injury (11). In the ageing brain, the accumulation of oxidative/nitrosative damage is well documented and such damage is implicated in the pathogenesis of several neurodegenerative diseases as well as acute cerebrovascular disorders (8, 17). Appropriate redox balance depends on the activity of antioxidant systems such as the thiol reducing system based round thioredoxin and peroxiredoxin (Prx), and that based on the synthesis and utilization of glutathione (1, 8, 22). These systems have widely documented neuroprotective effects in the CNS in the face of a variety of oxidative/nitrosative insults and disease models, and are able to catalyze a number of reactions that either neutralize ROS/RNS or reverse oxidative and/or nitrosative modification of proteins. Given their importance, factors that boost the activity or function of these antioxidant systems can have profound positive effects on neuronal health and

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vulnerability to insults. This forum issue focuses on recent advances in understanding what controls antioxidant defenses and oxidative/nitrosative stress in health and disease, in both the developing and mature brain.

Regulation of Antioxidant Defenses by Endogenous and Exogenous Neuroprotective Pathways

Despite the importance of neuronal antioxidant defenses, until recently relatively little was known about whether they are subject to dynamic regulation, or are simply a fixed function of neuronal type and age. This is an important issue, since any regulation could have the capacity to influence biological aging, or the progression of neurodegenerative processes. It is now clear that neuronal antioxidant defenses are indeed responsive to numerous external stimuli, and the molecular mechanisms underlying these changes are beginning to be elucidated. The antiapoptotic effects of nerve growth factor (NGF) are well documented; recent work, however, highlights its influence on intrinsic antioxidant defenses. NGF causes an upregulation of glutathione redox cycling, enhancing ROS detoxification (6). NGF also prevents Bcl-2-associated X-protein (Bax) translocation to the mitochondria, which itself is a cause of ROS generation, potentially due to Bax-induced cytochrome c release causing disruption of the electron transport chain (6). Interestingly, the redox state of the cell can also determine the degree to which cytochrome c itself can initiate apoptosis: the reduced form of cytochrome c cannot activate apoptotic protease activating factor 1 (Apaf-1), whereas the oxidized form can (2).

Synaptic activity has well-known antiapoptotic effects (9) but has recently been shown to also enhance intrinsic neuronal antioxidant defenses *via* synaptic NMDA-type glutamate receptor (N-methyl D-aspartate receptor [NMDAR]) signaling (11). The protective antioxidant effects of physiological synaptic NMDAR activity contrast with the harmful effects of chronic hyperactivation of all NMDARs, particularly extrasynaptic NMDARs (3, 10). The basis for this protection lies in part on a transcriptional program of gene expression centered on the thioredoxin/Prx system (21). Changes to other systems may also contribute: synaptic activity can also enhance the activity of the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (27). PGC-1α promotes the expression of a number of antioxidant genes through coactivation of the transcription factors that regulate them, which include nuclear respiratory factors and nuclear receptors such as the estrogen receptor (29). PGC-1α also controls processes such as glucose metabolism, energy homeostasis, adaptive thermogenesis, and mitochondrial biogenesis. As such, PGC-1α is strongly neuroprotective against oxidative/nitrosative insults as well as excitotoxicity. Interestingly, PGC-1α is also implicated in Huntington's disease pathogenesis, since its expression is directly suppressed by mutant huntingtin protein (mtHtt), and overexpression protects against mtHtt toxicity (4, 20). Conversely, PGC-1α is induced as part of several behaviors that induce adaptive stress response programs, such as calorie restriction and exercise. There is growing epidemiological evidence that caloric restriction, exercise, and cognitive stimulation reduce the risk of neurodegenerative disease, particularly Alzheimer's disease (30). Further, there is evidence that all these promote neuronal health in part through enhanced resistance to oxidative/nitrosative stress, potentially through upregulation of PGC-1α, neurotrophin expression, synaptic activity, or other mechanisms (30).

These other mechanisms may include adaptive responses to mild oxidative/nitrosative stress, long known to promote resistance or preconditioning of cells against subsequent toxic insults. Perhaps the major sensor of mild oxidative/nitrosative stress in this context is the Nrf2/Keap1 pathway (15). The transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) is a master regulator of antioxidant defenses and drug-metabolizing enzymes, controlling genes that contain a promoter sequence called the antioxidant response element (ARE). These comprise a group of cytoprotective and antioxidant genes that include multiple components of the thioredoxin pathway such as thioredoxin and sulfiredoxin, as well as key enzymes that regulate glutathione biosynthesis and utilization (15, 28). Under normal conditions Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1), and targeted for degradation (18). However, under oxidative/nitrosative stress conditions, a critical regulatory cysteine thiol on Keap1 reacts with ROS/RNS. This reaction promotes dissociation of Keap1 from Nrf2 and Keap1-mediated degradation of Nrf2 is thus inhibited, allowing the transcription factor to evade Keap1-mediated ubiquitination and accumulate in the nucleus where it activates ARE-containing genes (15). A variety of small molecules (including dietary phytochemicals) can also inhibit the action of Keap1 *via* S-alkylation of the same critical thiol, thus enabling the coordinated manipulation of brain antioxidant defenses with demonstrable protective effects in models of stroke and neurodegenerative disease (12, 24, 26, 31).

Influence of Acute and Chronic Neurological Disorders on Oxidative/Nitrosative Stress and Antioxidant Defenses

Not only is there a growing understanding of how protective signaling pathways can boost intrinsic antioxidant defenses, but also it is now clear that certain pathological processes can involve the specific inactivation of antioxidant enzymes, as well as the upregulation of ROS/RNS production. Stroke is a good example of a neurological disorder where molecular events take place that both enhance ROS/RNS production and diminish antioxidant defenses. Large quantities of ROS and RNS are produced during an episode of cerebral ischemia and subsequent reperfusion (3). A complex cascade of events leads to ROS and RNS generation at several points of the cascade from several sources, including reoxygenation, excitotoxicity, and inflammatory cytokine signaling. A key early source of neuronal superoxide during ischemia is overactivation of NADPH oxidase, and the discovery of this enzyme as a key mediator of oxidative stress reveals a potentially important therapeutic target (3). Concomitant with increased ROS generation, there is emerging evidence that Prxs become inactivated during an ischemic episode. Prxs have been shown to be protective in several models of ischemia *in vitro* and *in vivo* through overexpression studies (1). However, excitotoxic conditions *in vitro* and transient ischemia *in vivo* cause the hyperoxidative (or S-nitrosylation) inactivation of PrxII and PrxIII (5, 14, 21). In addition, inactivating phosphorylation of Prx II takes place during ischemia through activation of cyclin-dependent

kinase 5 (cdk5) (23). One could envisage that inactivation of Prxs makes it harder for the brain to cope with elevated ROS and RNS generation after stroke and possibly other neurodegenerative disorders.

A similar, though not identical, situation is emerging in Parkinson's disease (PD), where Prxs also exert cytoprotective effects in models of the disease (1). PrxII is subject to inactivation by cdk5-dependent phosphorylation and also by S-nitrosylation of its peroxidative cysteine residues (19). Physiological S-nitrosylation of proteins was first discovered in the context of regulation of the NMDA receptor by nitric oxide (16), but recent work suggests that it is a widespread occurrence that takes place in response to nitric oxide production and can have a harmful or beneficial outcome depending on the context and the protein targeted. In addition to PrxII, both Parkin and protein disulfide isomerase (PDI) become S-nitrosylated in human PD and in PD animal models (19). By altering the chaperone and isomerase activities of PDI, S-nitrosylation prevents the protective effect of PDI in the face of protein misfolding, whereas S-nitrosylation of Parkin affects its ubiquitin E3 ligase activity and protective function (19).

Many other S-nitrosylation events are known to occur in pathological scenarios, such as ischemia-induced S-nitrosylation of matrix metalloprotease-9, which activates the enzyme further, leading to neuronal loss (7, 25). Other targets for S-nitrosylation actually form part of a protective response to trauma, such as those targeting caspases, the NMDA receptor, and GOSPEL (GAPDH's competitor of Siah Protein Enhances Life) (25). The range and extent of targets for this modification are only now becoming clear.

Concluding Remarks

Despite the established link between the pathogenesis of neurodegenerative disease, stroke, and traumatic brain injury to oxidative/nitrosative stress, clinical trials of small molecule antioxidants and spin traps have met with limited success (13). There are myriad potential explanations for this, which include the challenge of maintaining sufficient levels of the drug in the brain to neutralize ROS/RNS as and when they appear. However, as described at length in this forum issue, the antioxidant and detoxification systems of the CNS are sophisticated and complex, with enzymes variously dedicated to neutralizing different types of ROS/RNS, and reversing harmful oxidative and nitrosative modifications. Often these reactions are specific for particular subcellular compartments. It may be asking too much of a single compound to be able to mimic more than a small subset of these reactions. An alternative antioxidant approach may be to concentrate on boosting the activity (or preventing the inhibition) of intrinsic antioxidant defenses. Researchers are learning more about the molecular events underlying the control of ROS/RNS-generating processes and antioxidant pathways, both in normal brains and in those affected by neurological disorders. These studies are revealing new therapeutic targets and hubs of control that may form the focus of future translational research.

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Abbreviations Used

Apaf-1 = apoptotic protease activating factor 1
ARE = antioxidant response element
Bax = Bcl-2-associated X-protein
Cdk = cyclin-dependent kinase
CNS = central nervous system
Htt = Huntingtin
Keap1 = Kelch-like ECH-associated protein 1
NGF = nerve growth factor
NMDAR = N-methyl D-aspartate receptor
Nrf2 = nuclear factor-erythroid 2-related factor
PD = Parkinson's disease
PDI = protein disulfide isomerase
PGC-1α = peroxisome proliferator-activated receptor-γ coactivator 1α
Prx = peroxiredoxin
RNS = reactive nitrogen species
ROS = reactive oxygen species

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