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CNS Peroxiredoxins and Their Regulation in Health and Disease

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Abstract

Oxidative stress is thought to be a contributing factor in many chronic neurodegenerative pathologies, as well as acute cerebrovascular disorders such as stroke. Peroxiredoxins are a family of antioxidant enzymes that reduce peroxides directly through the use of a redox active cysteine within their active site, which in the process becomes oxidized. In order to cycle back to the reduced state, many peroxiredoxins rely on thiol-dependent reduction by the ubiquitous antioxidant enzyme thioredoxin. Peroxiredoxins, together with thioredoxin and thioredoxin's own 'recycling enzyme', thioredoxin reductase, represent an antioxidant enzymic system of growing significance in the context of neuronal physiology and pathology. Overexpression, knockdown, and knockout approaches have demonstrated an important role for peroxiredoxins in protecting neurons from oxidative insults. It is also becoming clear that neuronal peroxiredoxins are subjected to post-translational modifications that impair function as part of disease pathology. Conversely, components of this pathway are also subject to dynamic upregulation such as *via* endogenous synaptic activity-dependent signaling and induction of the Nrf2-dependent Phase II response. As such, the thioredoxin-peroxiredoxin system represents a potential therapeutic target for central nervous system disorders associated with oxidative stress. *Antioxid. Redox Signal.* 14, 1467–1477.

Introduction

Regulation of cellular redox balance depends on the activity of antioxidant systems. Key among these are the thioredoxin (Trx) and glutathione thiol-based reducing systems, which are important reducers of many oxidative stressors such as peroxides (25, 101). The enzymic systems involved in the biosynthesis and utilization of glutathione are critical for maintaining correct redox balance in the brain (for discussion of the control of these systems, see Ref. 4). However, growing evidence suggests that the thioredoxin–peroxiredoxin system also makes an important contribution to neuronal antioxidant defenses.

The Trx family consists of cytoplasmic Trx1 and mitochondrial Trx2, as well as the more recently discovered microtubule-associated protein Txl-2 and transmembrane protein Tmx (65). These proteins all contain two redox-active cysteine residues (Cys-Gly-Pro-Cys) within their catalytic site, which in the reduced state exist in their –SH form. These cysteines are converted to the oxidized intramolecular disulfide bond state in a reaction that results in the reduction of a disulfide bond in the target protein. This oxidized form of Trx is then recycled back to its reduced form by thioredoxin reductase (TrxR), which uses NADPH as a cofactor (Fig. 1). Through its ability to reduce disulfide bonds in target proteins, Trx acts as a signaling molecule and thus can alter the activity of many target proteins, including apoptosis signal regulating kinase 1 (ASK1) and transcription factors such as nuclear factor-kappa B (NFκB) and p53 (32, 53, 59, 94). Trx is also an important part of cellular antioxidant defenses, as seen by its ability to protect cells against peroxide-induced cell death, and the fact that its inhibition promotes oxidative stress (101). Additionally, transgenic mice overexpressing human Trx have a longer lifespan than wild-type counterparts. They also display reduced neurodegeneration and oxidative damage following ischemia and a reduction in the amount of hippocampal neuronal loss in a kainate-induced seizure model (89, 90, 101).

While Trx can directly and independently quench singlet oxygen and scavenge hydroxyl radicals (18), its most widely studied antioxidant capacity has thus far centered on its cooperation with the peroxide scavengers peroxiredoxins (Prxs). This review focuses on recent advances in understanding the role and regulation of Prxs in the central nervous system (CNS). For a more comprehensive review on Trx cycle enzymes in the CNS, see Ref. 65.

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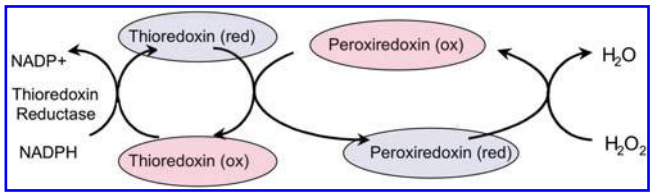

FIG. 1. The Trx-Prx antioxidant system. Schematic illustrating the flow of electrons from NADPH to hydrogen peroxide via thioredoxin reductase, thioredoxin, and peroxiredoxins, where *ox* and *red* refer to oxidized and reduced respectively, here and throughout (oxidized shaded *red*, reduced shaded *blue*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

The peroxiredoxin family

Prxs are a family of antioxidant enzymes that catalyze the reduction of peroxides (5, 96). Family members share the same basic catalytic mechanism in that they possess a redox-active peroxidatic cysteine residue that becomes oxidized by peroxides to cysteine sulfenic acid (-SOH, Fig. 2). This oxidized form of Prx must then be reduced/recycled back to the –SH form by reduced Trx, the precise mechanism of which differs amongst the Prx subfamilies. The 2-Cys Prxs are the predominant Prx subfamily, comprising Prx I-IV (5, 96). 2-Cys Prxs possess a 'resolving cysteine' residue as well as a peroxidatic one; the former reacts with the oxidized peroxidatic Cys-OH to form a disulfide bond. In the typical 2-Cys Prxs (Prx I–IV), the resolving Cys and catalytic Cys are on different molecules and so these exist as functional homodimers (Fig. 2). In contrast, Prx V is an atypical 2-Cys Prx that exists as a monomer with both the resolving and catalytic Cys residues on the same molecule. A distinct class of Prxs which in mammalian cells only comprises one member is the 1-Cys Prx subtype (Prx VI). The 1-Cys Prx do not contain a resolving cysteine and cannot be reduced by Trx, but may instead be reduced by other agents, potentially glutathione or cyclophilin.

The reportedly low catalytic rates for Prxs led to the suggestion that Prxs would be unable to compete with catalase or glutathione peroxidases for cellular peroxide, despite evidence of the antioxidative properties of Prxs. This apparent paradox stemmed from an underestimation of the peroxidase rates of mammalian Prx II, due to an indirect calculation by measuring NADPH oxidation rates, which actually reflect regeneration by the Trx system. When peroxide levels are high, it is clear that the Trx system cannot keep up. In fact, when rates of mammalian Prx were measured directly (66), they were found to be 100-fold greater than previously thought, similar to rates recently found in PrxI and II from *S. cerevisiae* (62). Since Prxs are relatively abundant, they are also likely to play an important antioxidant role in mammalian *in vivo* systems.

Expression patterns of the various Prxs have not been extensively studied in the CNS, however, some potentially important cell-type specific differences have been reported. Mouse forebrain neurons have been shown to express Prxs II, III, IV, and V, while astrocytes and microglia express only Prx VI and Prx I, respectively (37). In the human brain, neurons in the cerebral cortex, cerebellum, basal ganglia, substantia nigra, and spinal cord, were found to express Prx II but not Prx I while astrocytes in the same areas expressed Prx I but not Prx II (76). Moreover, Prx II was not expressed uniformly in all human neurons: smaller neurons (*e.g.*, cerebellar granule neurons) displayed little expression, while large neurons (*e.g.*, hippocampal pyramidal and Purkinje neurons) expressed Prx II more strongly (76).

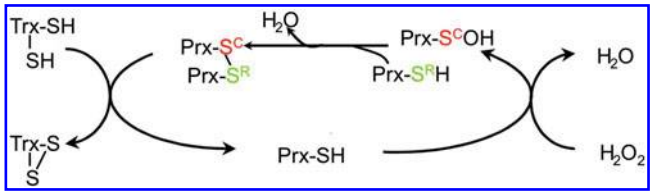

FIG. 2. Mechanism of conventional 2-Cys Prxs. The peroxidatic, catalytic cysteine residue of Prx (S^c, in *red*) reduces peroxide, and is in turn converted to cysteine sulfenic acid (S^COH). The resolving cysteine (S^R, in *blue*) of another Prx then forms a disulfide bond with Prx-S^COH, eliminating H_2O. This intermolecular disulfide bond is in turn reduced by Trx, whose 2 cysteine active site is converted to an intramolecular disulfide bond (reduced by Trx reductase). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Hyperoxidation of Prxs and its reversal by sulfiredoxin

Under increased oxidative stress, Prx-SOH can sometimes be further oxidized by peroxide to sulfinic (-SO_2H) acid, causing inactivation of peroxidase activity (72). Prx-SO_2H is not a substrate for the resolving cysteine and cannot be reduced by Trx (Fig. 3). As such, Prx hyperoxidation to Prx-SO_2H was thought to be irreversible. Subsequently, it was found that Prx-SO_2H can be reduced back to the catalytically active thiol form by two ATP-dependent reductases, sulfiredoxin (6, 11) and sestrin 2 (9). However, recent doubt has been cast on the ability of sestrin 2 to catalyze this reaction (73), although others have shown a role (20). Sulfiredoxin, which was initially characterized in yeast (6) and then in mammalian cells (72), acts by catalyzing the ATP-dependent formation of a sulfinic acid phosphoric ester on Prx (72) which is then reduced by thiol equivalents such as Trx. Thus, sulfiredoxin acts as an important adjunct of the Trx–Prx system, preventing bursts of reactive oxygen species (ROS) from

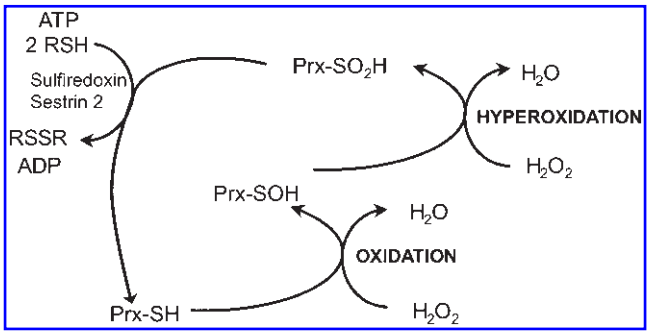

FIG. 3. Hyperoxidation of Prxs and their reduction. See text for details.

sending Prxs down a *cul-de-sac* of permanent hyperoxidative inactivation (Fig. 3).

Prxs in Aging and Neurological Disorders

Since both aging and many neurodegenerative diseases are associated with oxidative stress, several studies have addressed the question of whether Prx expression is altered under these conditions. Perturbation in the expression level of several Prxs has been reported in human neurodegenerative disease, and animal models thereof (65). Prx II is elevated in Alzheimer's disease (AD) by a mechanism linked to the association/binding of amyloid beta protein (Aβ) to amyloid binding alcohol dehydrogenase (40, 99)). Prx II expression is also increased in the cortex of AD, Parkinson's disease (PD), and Down's syndrome (DS) patients, while levels of mitochondrial Prx III were found to be lower in DS and PD (40, 44).

In the aging brain, increased expression of Prx II has been observed in the mouse hippocampus (41), although a reduction in Prx II expression with age has been reported in humans (13). It is not easy to draw strong conclusions from these expression studies, since upregulation can be interpreted as a response to oxidative stress, and downregulation as a contributing factor to the underlying stress. However, more recent studies have shed light on the role of Prxs in certain neurological disorders (see below), both as a protecting force and as a target for disease-associated signaling pathways.

Prxs in the aging brain

As initially proposed by Harman (29), the free radical theory of aging contends that biological aging is associated with a progressive accumulation of oxidative damage to components of cells, with consequent decline of normal function. Such accumulation has been shown in the aging human brain (55) in the form of oxidative modification of DNA (*e.g.*, 8-hydroxy-2-deoxyguanosine (8-OHdG)), lipid peroxidation (*e.g.*, assayed by measuring markers such as 4-hydroxy-2-nonenal), and protein alteration (*e.g.* protein carbonylation). In addition to these general markers of oxidative damage, oxidative modification of specific cellular components, such as specific gene promoters (54), may also mechanistically contribute to the aging process. Recent studies, such as the finding of age-associated PrxI carbonylation in the rat cortex (95), have implicated Prxs as targets of age-related modifications. Moreover, levels of hyperoxidized Prxs have also been reported to be age dependent. For example Prx-SO_2H was found to be initially elevated in young gerbils, prior to a decline in adulthood and followed by a significant re-elevation with old age (100). Hyperoxidized Prxs have also been reported in the periphery in organs such as the liver (58).

Studies in lower organisms indicate that Prxs are important in combating the aging process and in increasing lifespan. In Drosophila, homologues of Prx II and Prx V are associated with promoting longevity: overexpression of Prx V was found to increase lifespan, while Prx V-deficient flies were more vulnerable to oxidative stress and apoptosis and suffered a shortened life (69). Of note, the tissue-specific pattern of spontaneous apoptosis observed in Prx V-deficient flies was similar to that observed in wild-type flies treated with paraquat (*i.e.*, cells/tissues particularly vulnerable to oxidative insults). Neuronal knockdown of the Prx II homologue Jafrac1 was also found to shorten lifespan, while its overexpression, or that of human Prx II, increased lifespan (48).

In mice, Prx II plays an important role in suppressing oxidative damage, particularly in aged individuals. Compared to wild-type mice, hippocampal tissue from Prx II-deficient mice displays elevated levels of both protein carbonylation and malondialdehyde (a marker of lipid peroxidation). However, this difference is only found in mice 6 months of age or older: at 3 months, no difference from wild type is present (41). Mitochondria are key targets of ROS attack and mitochondrial dysfunction is thought to be an important contributor to oxidative stress-induced cellular dysfunction. Consistent with this, age-associated mitochondrial dysfunction is observed in Prx II-deficient mice, as measured by oxidized mitochondrial DNA and lowered Complex IV and V activity, as well as a reduction in the number of intact mitochondria (41). In young mice, however, mitochondrial function is unimpaired. Prx II-deficient mice also exhibit age-linked deficits in spatial learning and long-term potentiation (LTP) (41). Hippocampal slices from Prx II-deficient mice exhibited a far steeper age-dependent decline in LTP than wild-type. This is consistent with the notion that accumulation of oxidative damage contributes to cognitive decline due to neuronal dysfunction (as opposed to outright death). Interestingly, the age-associated decline in plasticity, cognitive performance, and mitochondrial integrity observed in Prx II-deficient mice is reversed by dietary supplementation of the free radical scavenger vitamin E (41). This strongly indicates that the effects of Prx II deficiency are due to its role as an antioxidant, as opposed to other Prx-II dependent functions.

Prxs in Alzheimer's and Parkinson's Diseases

There is growing evidence that Prxs not only have the capacity to protect against certain disease-causing agents (Fig. 4) but are also targets for disease-associated signaling pathways. Several different types of Prx modification have been reportedly associated with AD. Prx II exists in a more oxidized state in AD than in control individuals, potentially indicating that the AD pathology places a greater oxidative demand on

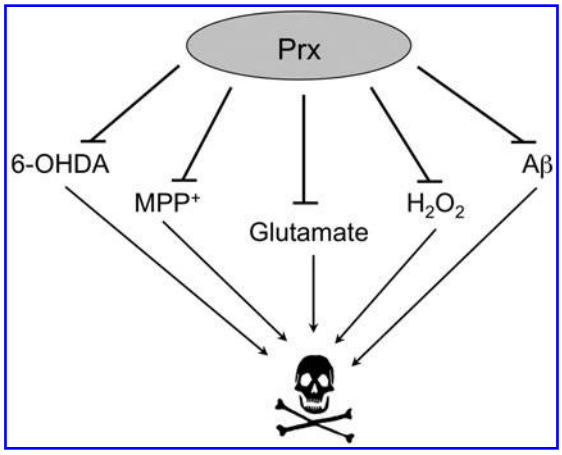

FIG. 4. The Prx family protects against a variety of cellular insults. Schematic displaying neurotoxic insults known to induce cell death/damage, against which Prx have been shown to be protective. See text for details.

the Prx system (17). Prx II has also been identified as a target for nitration in AD, although the functional consequence of this modification remains unclear (71). Prx I, thought to be predominantly expressed in astrocytes, is carbonylated in AD brains (95). Overexpression of both Prx I and Prx II protects primary neurons against Aβ toxicity (17, 99), although the relevance of Aβ toxicity to AD pathology is not clear-cut. It is also interesting to note that the oxidation state of Prxs II and VI in the blood has been found to be elevated in AD patients (102). Given that Prx II is the third most abundant protein in erythrocytes, any CNS disease-induced modification of blood Prx II could represent a potentially attractive biomarker.

Perhaps the most active area of research into the role of Prxs in neurodegenerative diseases is in the PD field. PD is primarily caused by the death of dopaminergic neurons within the substantia nigra (SN), an event associated with increased oxidative stress and also mitochondrial dysfunction. While the trigger for these processes is not fully understood, dopaminergic neurons of the SN are particularly vulnerable to oxidative stress and mitochondrial toxins, which have been used to model cell death *in vitro* and to induce the Parkinsonian phenotype in rodent models of the disease. Recent studies have unveiled an important role for Prx II in the PD pathology, both in protecting dopaminergic neurons from oxidative stress and also as a target of the PD pathoprogression.

Calpain-mediated activation of cyclin dependent kinase 5 (Cdk5) is known to be an important mediator of mitochondrial toxin-evoked dopamingergic neuronal death in an experimental model of PD (81). Cdk5's ability to mediate cell death has since been shown to occur via phosphorylation and subsequent inactivation of Prx II (68), leading to an impairment of Prx II's peroxidase activity (Fig. 5). A subsequent study confirmed the peroxidase-inhibiting effects of Cdk5-dependent phosphorylation of Prx II and also Prx I (87). Transduction of a nonphosphorylatable form of Prx I or II protected HT22 cells against glutamate toxicity, while inhibition of Cdk5 prevented glutamate-induced ROS accumulation and cell death, and also preserved Prx peroxidase activity (87). In agreement with this, knockdown of Prx II has been found to enhance oxidative stress and vulnerability to the dopaminergic neurotoxin MPP+ (1-methyl-4-phenylpyridinium), while overexpression of Prx II (but not a phospho-mimetic mutant) is protective. Finally, Prx II phosphorylation is observed in substantia nigral neurons of human PD postmortem tissue, but not in non-PD subjects.

Phosphorylation is not the only inactivating modification important for PD. Excessive nitric oxide (NO) production, leading to protein S-nitrosylation, has also been implicated in the progression of several neurodegenerative disorders and pathological processes such as protein misfolding (60). For example, S-nitrosylation of Parkin and protein-disulfide isomerase (PDI, an endoplasmic reticulum (ER) chaperone) may contribute to the accumulation of misfolded proteins in PD and other disorders (16, 52, 60, 93). Recently, Prx II was also shown to be a target for S-nitrosylation (Fig. 5): NO can trigger Prx II S-nitrosylation on at least two cysteine residues, serving to strongly inhibit the peroxidase activity of the enzyme (21). Thus, while expression of Prx II protects dopaminergic cells against peroxide-induced death, prior exposure to a NO donor blocks this protection. S-nitrosylation of Prx II can be induced by the mitochondrial toxins MPP+ and rotenone, agents known to induce a Parkinsonian phenotype *in vivo*. Importantly, elevated levels of nitrosylated Prx II were found in the brains of PD patients (but not AD), demonstrating that this inhibitory modification has direct relevance to the human condition.

Prxs in stroke

Brain ischemia and subsequent reperfusion leads to the generation of superoxide and other ROS, as well as substantial oxidative damage in both the infarct core and penumbra (55). Damage due to acute ischemic stroke is a complex cascade of excitotoxicity, ROS generation, and inflammatory responses, with several mechanisms and cell types being implicated in the generation of ROS (8). In neurons and other cells, anoxia can cause an accumulation of reducing equivalents within the electron transport chain that can lead to a burst of ROS production following reperfusion (3, 8, 55). In addition, ischemia leads to an accumulation of extracellular glutamate due to synaptic release and impaired/reversed uptake mechanisms (10, 74), resulting in overactivation of N-methyl-D-aspartate receptors (NMDARs) (15). Excessive NMDAR activation results in substantial superoxide generation through activation of NADPH oxidase (12, 86). Toxic levels of NO are also produced through overactivation of the Ca^{2+}-dependent neuronal NO synthase (22). Accumulating evidence suggests that

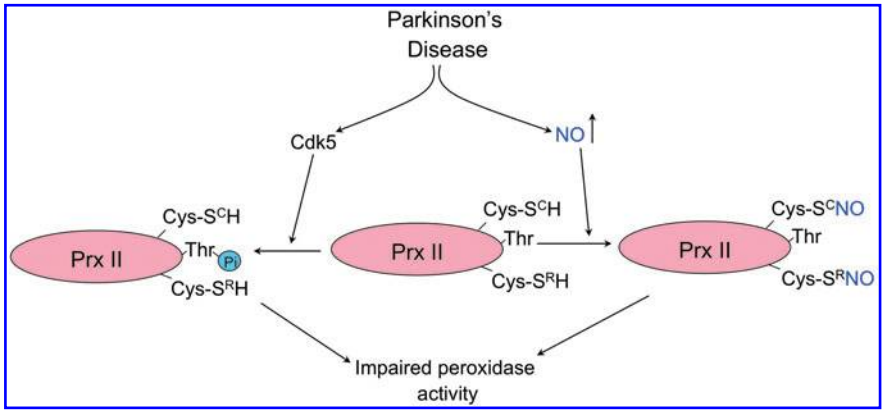

FIG. 5. Parkinson's disease is associated with covalent modifications on Prx II. Both phosphorylation and S-nitrosylation of Prx II have been described within the PD pathology, leading to an inhibition of the peroxidase activity of Prx II (21, 68, 87). Transduction of a nonphosphorylatable form of Prx I or II protected HT22 cells against glutamate. Thr refers to threonine-89. NO moiety is *highlighted in blue*. Phosphorylation is *highlighted in turquoise*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Prxs exert a protective effect in the face of an ischemic challenge, or glutamate excitotoxicity, but can also be subject to catalytic inactivation by the very same events (Fig. 6).

Upregulation of mitochondrial Prx III and Trx2 in the hippocampus has been observed following an episode of transient cerebral ischemia in rodents. Furthermore, intracerebroventricular administration of Prx III or Prx III and Trx2 combined was neuroprotective in the same model (33). *In vitro*, Prx III overexpression is similarly protective of hippocampal neurons against excitotoxic insults (30). Prx II also plays a key role in protecting neurons against ischemic/excitotoxic insults, but like mitochondrial Prx II it is also a target for catalytic inactivation. Overexpression of Prx II protects cortical neurons against oxygen-glucose deprivation (7) and protects cerebellar granule neurons against glutamate excitototoxicity (70).

We recently investigated whether an episode of ischemia-reperfusion triggered sufficient ROS production that Prxs become hyperoxidized (63). Adult mice were subjected to a transient middle cerebral artery occlusion (MCAO) and cortical extracts were examined 4 hours post-reperfusion, a time point prior to infarct development. Increased levels of hyperoxidized Prxs were observed in the MCA territory (cortex and striatum) following ischemia/reperfusion, compared to equivalent samples from sham-operated individuals (Fig. 6). Of note, the profile of hyperoxidation is subtly different from that of simple *in vitro* H_2O_2 treatment: mitochondrial Prx III is overoxidized to a greater extent by ischemia/reperfusion than by H_2O_2 treatment. Exposure of cortical neurons to glutamate is sufficient to induce Prx hyperoxidation, although to a degree which is less pronounced than either *in vivo* ischemia or peroxide treatment *in vitro* (50). The strong hyperoxidation of Prx's peroxidatic cysteines induced by ischemia or excitotoxicity is in contrast to PD models, where hyperoxidation is weak (21). Indeed, S-nitrosylation of Prx's peroxidatic cysteines observed in PD will of course prevent any hyperoxidation of the same residue. As such, the issue of whether S-nitrosylation or hyperoxidation take place could be down to the relative levels of NO vs. peroxides. In acute ischemia/reperfusion the latter may dominate, while NO accumulation in the absence of high levels of peroxide may favor the former.

In addition to hyperoxidative inactivation of Prxs, inactivating phosphorylation also takes place (Fig. 6). Glutamate triggers cdk5-dependent Prx II phosphorylation in cerebellar granule neurons (70) and expression of wild-type Prx II, but not a phospho-mimetic mutant, protects against glutamate toxicity and global ischemia-induced hippocampal damage. Thus, it is apparent that the Prxs represent an emerging and potentially important therapeutic target in stroke.

Control of the Trx–Prx System by Synaptic NMDAR Activity

Synaptic NMDAR activity is neuroprotective and boosts antioxidant defenses

In contrast to the adverse effects of excessive NMDAR activation, physiological levels of synaptic NMDAR activity can be important for neuronal survival, as outright blockade leads to deleterious effects (27). Elimination of NMDA receptor activity *in vivo* causes widespread apoptosis and enhances trauma-induced injury in developing neurons (1, 24, 34, 57, 67). In the adult CNS, NMDAR blockade exacerbates neuronal loss when applied after traumatic brain injury and during ongoing neurodegeneration (35), and prevents the survival of newborn neurons in the adult dentate gyrus (91). Aside from stimulus intensity, the location of the NMDAR may also profoundly affect resulting signaling cascades. Developing neurons have sizeable pools of NMDARs at extrasynaptic, as well as synaptic locations (56, 92), which each display distinct signaling patterns. Ca^{2+} influx occurring solely as a result of intense synaptic NMDAR activation is well tolerated by cells, whereas equivalent activation of extrasynaptic NMDARs, either on their own or in the presence of coincident synaptic NMDAR activation, is poorly tolerated, triggering mitochondrial membrane depolarization and cell death (28, 83). The survival signaling originating from synaptic NMDAR activation has been recapitulated *in vitro* in neuronal cultures (26, 31), allowing scientists to study the underlying signaling events. Pro-survival signaling from the synaptic NMDAR has been shown to involve post-translational modifications of proteins as well as *de novo* gene expression. Different signals may have varying levels of importance depending on the initiating circumstance (27; Fig. 7). For example, protection against nonoxidative apoptotic insults involves suppression of components of the intrinsic apoptosis pathway and an upregulation of anti-apoptotic pro-survival genes (45, 49, 64, 104).

The vulnerability of cortical neurons to peroxide-induced death was found to be regulated by synaptic activity acting via NMDAR signaling ((63), see also (51)). Neurons that are experiencing (or that had recently experienced) higher levels of synaptic NMDAR activity were far more likely to withstand the oxidative insult than electrically quiet neurons. This

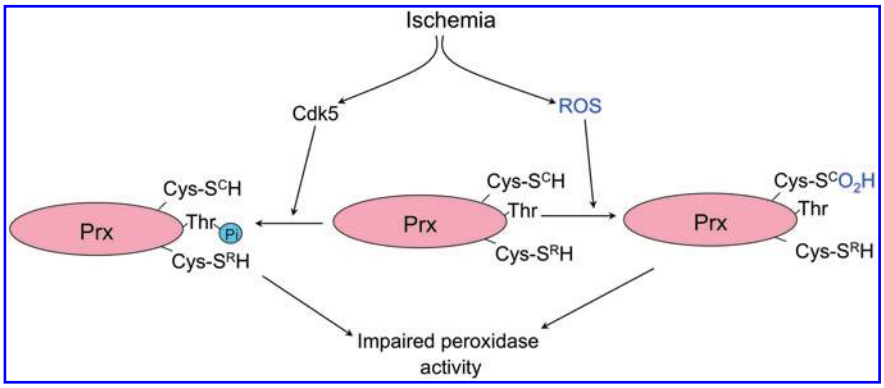

FIG. 6. Ischemia is associated with covalent modifications on Prxs. Both phosphorylation and hyperoxidation of Prxs has been described following episodes of ischemia (63, 70), which cause an inhibition of the peroxidase activity of Prxs. ROS and cysteine sulfinic acid moiety *highlighted in blue*. Phosphorylation *highlighted in turquoise*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

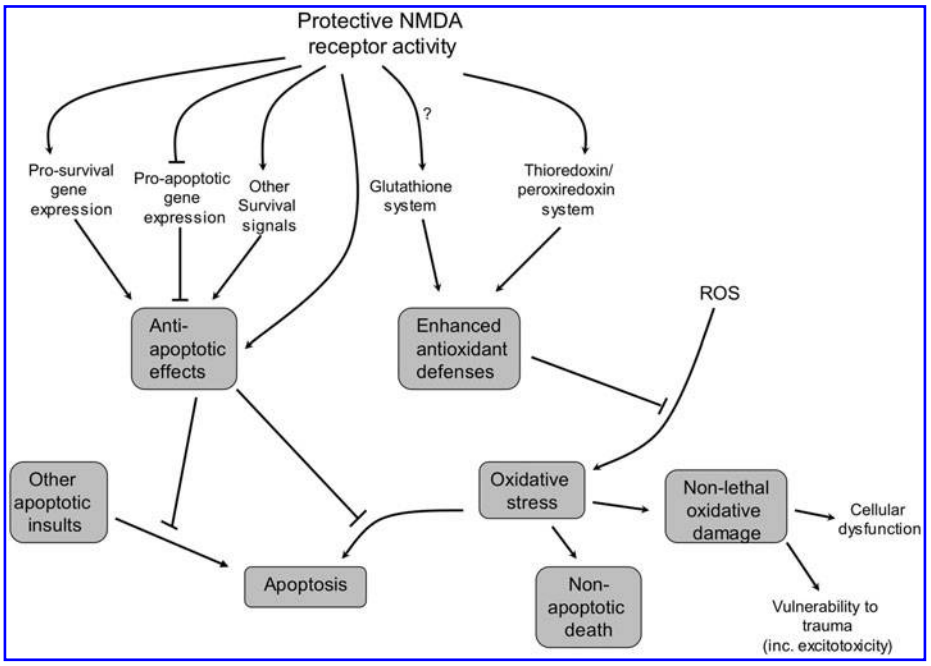

FIG. 7. Synaptic NMDA receptor activity both boosts intrinsic antioxidant defenses and inhibits the apoptotic pathway. See text for details.

is in line with findings that neurons experiencing complete NMDAR blockade are highly vulnerable to peroxide-induced apoptosis *in vitro*, and that NMDAR blockade *in vivo* promotes neuronal apoptosis associated with markers of oxidative damage (63). It is not completely clear as to why synaptic activity, acting via Ca^{2+} signalling, should act to boost antioxidant defenses. Given that synaptic activity and activity-dependent plasticity place high energy demands on a neuron, which are largely met through oxidative phosphorylation (a process that generates ROS), it is conceivable that this event helps to protect against ROS. Thus, active neurons would ordinarily require stronger antioxidant defenses than inactive neurons, in order to maintain the correct redox balance.

Synaptic activity targets several genes in the Trx–Prx pathway

Investigations into the mechanism behind this divergent response in electrically quiet versus active neurons revealed that electrically quiet neurons display higher levels of hyperoxidized Prxs when challenged with peroxide, indicative of an overwhelmed Trx–Prx system. In contrast, electrically active neurons did not display such an elevation, suggesting that the capacity of this system was in some way being enhanced.

We found that synaptic activity enhanced Trx activity, but paradoxically, that levels of Trx were unaltered (as were Prx and Trx-reductase levels). This observation was explained by the fact that synaptic activity triggers the transcriptional suppression of the Trx inhibitor, thioredoxin interacting protein (Txnip). Txnip interacts with the reduced form of Trx and inhibits its biochemical activity, sensitizing a variety of cell types to H_2O_2-induced death (78, 101). Under conditions of NMDAR blockade where Trx activity was lowest, levels of Txnip were elevated and it was found to associate *in vivo* with Trx. In contrast, elevated synaptic activity, strongly suppresses Txnip expression (Fig. 8). Overexpression of Txnip was sufficient to reduce activity-dependent protection against peroxide-induced neuronal apoptosis. We found that suppression of Txnip is mediated by a class of transcription factors called Forkhead box O (FOXO). Three of the four FOXO isoforms (FOXO1, FOXO3, and FOXO4) are regulated by Akt-dependent phosphorylation, triggering their export from the nucleus (75). Synaptic activity turns off *Txnip* transcription by inducing phosphoinositide 3-kinase (PI3K), which then activates Akt, triggering FOXO1 phosphorylation, dissociation from the *Txnip* promoter, and export from the nucleus (Fig. 8 and (63)). Of note, we subsequently found that FOXO1 itself is regulated by FOXOs (2).

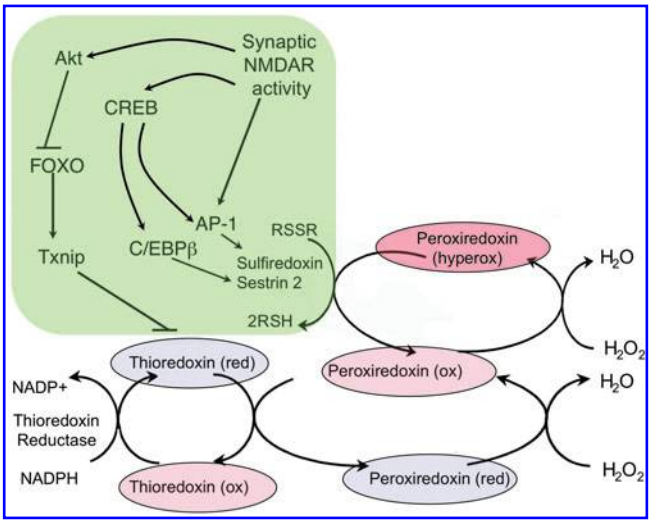

FIG. 8. Synaptic NMDA receptor activity controls the expression of components of the Trx–Prx system. See text for details. Oxidized state is shaded *red*, reduced state is shaded *blue. Green area* highlights the activity-dependent modification of the system. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Neuronal activity was found not only to prevent Prx hyperoxidation, but also to enhance the recovery of hyperoxidized Prxs following a brief high pulse of peroxide. This enhanced reduction of hyperoxidized Prxs was associated with transcriptional induction of the two genes, sulfiredoxin and sestrin 2 (Fig. 8), whose products are reported to mediate this reaction (6, 9, 11). The induction of sulfiredoxin is mediated primarily through two AP-1 sites, and partly via nuclear factor erythroid 2-related factor (Nrf2) (84), while sestrin 2 was activated via two C/EBPβ sites (63). While we did not address which of sestrin 2 or sulfiredoxin was responsible for reducing Prx hyperoxidation, we recently showed that specific induction of sulfiredoxin is sufficient to prevent Prx hyperoxidation in neurons (84), suggesting that sulfiredoxin is likely to be the most important. This is particularly relevant as recent doubt has been cast on the ability of sestrin 2 to catalyze this reaction [(73), though see (20)]. In any case, induction of one (or both) of these genes serves to cooperate with the suppression of Txnip in order to boost neuronal antioxidant defenses. Interestingly, the above described changes in gene expression do not account for the entire antioxidant effect of synaptic NMDAR activity. Work in our laboratory is currently ongoing to investigate the contribution of another major antioxidant system also subject to activity-dependent enhancement: that centred on glutathione.

Control of the Trx–Prx System by Nrf2 Activators

A known defense against oxidative insults is the induction of a group of genes encoding antioxidative and drug-metabolizing enzymes (39). These genes are induced by a variety of small thiol-active molecules and electrophiles (39, 61). Transcriptional regulation of this group of genes is mediated by a *cis*-acting promoter element termed the antioxidant response element (ARE), which recruits the transcription factor Nrf2 as a heterodimer with small Maf proteins (103). Nrf2 levels are constitutively low due to being targeted for degradation by Kelch like ECH-associated protein (Keap) 1 (Fig. 9). Under conditions of oxidative stress, Nrf2 degradation is slowed, allowing for Nrf2 accumulation in the nucleus and subsequent activation of ARE-containing genes, with a net antioxidative effect (61). Small molecule activators of Nrf2 also act by interfering with Keap1-mediated degradation. Activation of Nrf2 and induction of ARE-driven defences is implicated in protection against a variety of diseases in many organs and tissues, including autoimmune, neurodegenerative and cardiovascular diseases, as well as cancer and ischemia (23, 46, 80, 103).

Upregulation of Nrf2 activity in Drosphila increases lifespan and resistance to oxidative stress (88). In mammals, gene expression programs induced by Nrf2 can protect many different organs against a variety of traumas. For example, Nrf2 protects lung tissue against hyperoxic injury (14), and the liver against paracetamol-induced hepatotoxicity (19). Nrf2 is the primary molecular target of cancer chemopreventive blocking agents (23) which in general act by preventing carcinogens from forming adducts with DNA that lead to mutations. The boosting of intrinsic cellular antioxidant defenses of the cell by Nrf2 activation is believed to be an important mediator of these protective effects, as is the co-ordinated induction of detoxification enzymes such as glutathione S-transferases and NAD(P)H:quinone oxidoreductase 1 (NQO1).

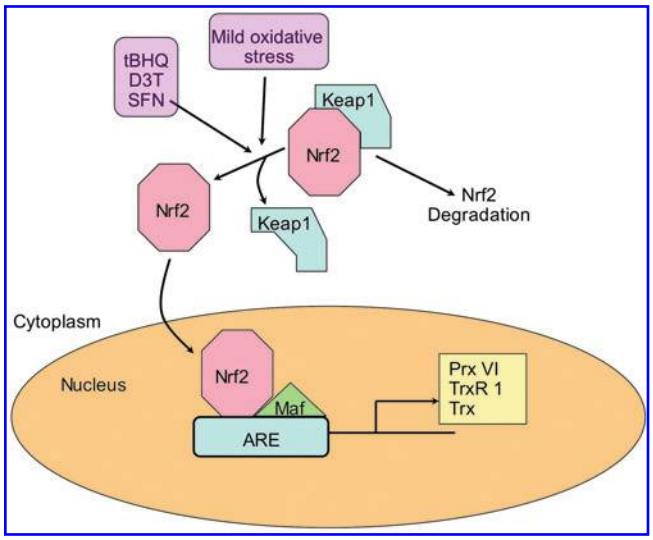

FIG. 9. Nrf2 promotes the expression of components of the Trx–Prx system. Both mild oxidative stress and small molecule activators of Nrf2 (*pink*) block the Keap1- and ubiquitin-dependent degradation of Nrf2, causing Nrf2 to be released by Keap1 allowing for translocation to the nucleus and subsequent transcriptional activation of genes containing an antioxidant response element (ARE). D3T: 3H-1,2-dithiole-3-thione; SFN: Sulforaphane; tBHQ: tert-butylhydroquinone. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

In the central nervous system, Nrf2 has been suggested to be a therapeutic target in excitotoxic disorders such as stroke and seizure, as well as in neurodegenerative disease (46, 80). Nrf2-overexpressing glial cells strongly protect surrounding untransfected neurons (36, 43, 79) and expression and/or activation of Nrf2 in neurons is also strongly protective (77, 84). Since the Nrf2 pathway is activated by mild oxidative stress, its upregulation has been reported following trauma. For example, traumatic brain injury activates endogenous Nrf2 in the CNS (97) and ischemia activates known Nrf2 target genes. It is possible that this pathway may therefore form part of an intrinsic protective response, or even contribute to processes such as ischemic preconditioning (a possibility currently under investigation in our lab).

The program of gene expression induced by Nrf2 is well-placed to combat the actions and the production of a variety of free radicals (23, 61, 103). Nrf2 activates central components of the glutathione (GSH) system, including those needed for GSH biosynthesis (glutamate-cysteine ligase) and recycling (glutathione reductase). Other targets include Ferritin which sequesters Fe(II) and can thus restrict hydroxyl radical-generating Fenton chemistry. Also, Nrf2 target gene heme oxygenase-1 (HMOX1) degrades the pro-oxidant heme molecule, generating bilirubin as a breakdown product, which can then react directly with, and neutralize, superoxide, hydroxyl and peroxynitrite radicals (85).

Nrf2 also promotes the expression of a number of components of the Prx–Trx pathway (Fig. 9), including Trx itself, as well as Trx reductase 1, Prx I, and Prx VI (42, 47, 79). We recently found a new member of the Nrf2 target gene family in sulfiredoxin (82, 84). In both neurons and glia, Nrf2

expression and treatment with chemopreventive Nrf2 activators, including D3T, sulforaphane and tBHQ, leads to an upregulated expression of sulfiredoxin via a consensus ARE (82, 84). An interesting facet of sulfiredoxin regulation is that its Nrf2- and AP-1- responsiveness is contained within the same sequence (82). The proximal conserved AP-1 site is contained at the heart of the ARE. Several AREs such as those in the promoters of human *NQO1* and *HMOX1* contain AP-1 like sequences and can respond to AP-1-activating stimuli, as well as Nrf2 (61). The composite ARE/AP-1 site on the sulfiredoxin promoter enables it to respond both to small molecule Nrf2 activators (84) as well as synaptic activity (63).

Consistent with the powerful effect of Nrf2 on the Trx–Prx system, the well-characterized Nrf2 activator, the chemopreventive agent 3H-1,2-dithiole-3-thione (D3T), also protects neurons against oxidative stress and inhibits the hyperoxidation of Prxs in response to an oxidative insult (84). Thus, targeting the Nrf2 pathway boosts the capacity of most major antioxidant systems, including the Trx–Prx system. Given that this system is impaired in pathological scenarios such as stroke, AD, and PD, it is unsurprising that exploiting the Nrf2 pathway to inhibit acute and chronic neurodegenerative processes is an area of active research (38, 46, 80, 98).

Conclusion

Despite the undisputed central role of the glutathione system in the antioxidant defenses of the brain, it is becoming clear that Prxs are also important. The differential expression of the six Prxs with regard to brain region, cell type, and subcellular location is suggestive of specialized antioxidant functions that are only now beginning to emerge. Nevertheless, it is clear that the cytoprotective and antioxidant activity of the Prxs are both directly and indirectly affected by numerous physiological and pathological signaling events within neurons.

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Abbreviations Used

AD = Alzheimer's disease
ARE = antioxidant response element
ASK1 = apoptosis signal regulating kinase 1
Cdk = cyclin dependent kinase
CNS = central nervous system
Cys = cysteine
DS = Down's syndrome
ER = endoplasmic reticulum
FOXO = forkhead box O
GSH = glutathione
HMOX1 = heme oxygenase-1
Keap = kelch like ECH-associated protein 1
MCA = medial cerebral artery
MPP+ = 1-methyl-4-phenyl-pyridinium
NFκB = nuclear factor-kappa B
NMDAR = N-methyl D-aspartate receptor
NO = nitric oxide
NQO1 = NAD(P)H:quinone oxidoreductase 1
Nrf2 = nuclear factor erythroid 2-related factor
PD = Parkinson's disease
PDI = protein-disulfide isomerase
Prxs = peroxiredoxin
ROS = reactive oxygen species
Trx = thioredoxin
TrxR = thioredoxin reductase
Txnip = thioredoxin interacting protein

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