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Forum Review

Essential Cellular Regulatory Elements of Oxidative Stress in Early and Late Phases of Apoptosis in the Central Nervous System

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ABSTRACT

The generation of reactive oxygen species and subsequent oxidative stress in the central nervous system is now considered to be one of the primary etiologies of a host of neurodegenerative disorders, such as Alzheimer disease, Parkinson disease, and cerebral ischemia. On a cellular level, oxidative stress leads to an apoptotic early phase that involves cellular membrane phosphatidylserine (PS) exposure and a late phase that pertains to the degradation of genomic DNA. The translocation of membrane PS from the inner cellular membrane to the surface is a critical component for both microglial activation and cellular disposal of injured cells. During oxidative stress, this early phase of apoptosis is intimately controlled by neuronal PS exposure and microglial PS receptor expression. The late phase of apoptosis that involves a loss of genomic DNA integrity can result as a function of an ill-fated attempt to enter the cell cycle in postmitotic neurons. By using a cascade of pathways that involve cysteine proteases to modulate programmed cell death, protein kinase B (Akt) surfaces as a key regulatory element of both extrinsic pathways of inflammation and intrinsic pathways of cellular integrity. Further understanding of the cellular mechanisms modulating neuronal cellular integrity and phagocytic cell disposal during oxidative stress may form the basis for the future development of cytoprotective strategies in the nervous system. *Antioxid. Redox Signal.* 6, 277–287.

ROLE OF OXIDATIVE STRESS IN NEURODEGENERATIVE DISORDERS

FREE RADICALS AND SIMILAR AGENTS are classified together as compounds that come under the description of reactive oxygen species (ROS). ROS include oxygen free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite. Overproduction of ROS in cells leads to oxidative stress that ultimately results in cellular damage (77, 80). ROS are capable of producing cell damage through cell membrane lipid destruction (71), cleavage of DNA (36), production of protein carbonyl derivatives and the formation of nitrotyrosine (1), and the blockade of mitochondrial respiration (84).

The brain is extremely sensitive to oxidative stress due to its enriched amount of unsaturated fatty acid, higher oxygen metabolic rate, and its weaker defense system against ROS. ROS can attack polyunsaturated fatty acids that lead to lipid peroxidation (71). In addition, ROS can alter DNA integrity through the hydroxylation of guanine and the methylation of cytosine, which is critical in the regulation of DNA function (36). Amino acids are another target of ROS that subsequently result in protein oxidative damage with the production of protein carbonyl derivatives and the formation of nitrotyrosine (1). Other ROS, such as peroxynitrite, have been demonstrated to inhibit complex enzymes in the electron transport chain of the mitochondria, resulting in the blockade of mitochondrial respiration in neurons (84).

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Oxidative stress has been found to play a critical role in the progression of neurodegenerative diseases. There exist several lines of evidence that suggest oxidative stress may be a principal precipitant of neuronal injury, such as in Alzheimer disease. Alzheimer disease is characterized by the presence of neurotic plaques and neurofibrillary tangles. The β-amyloid (Aβ) deposition that constitutes the plaques is composed of a 39–42-amino acid peptide. Aβ is the proteolytic product of amyloid precursor protein (APP). Large soluble fragments (APPs) that are the result of APP cleavage within its Aβ domain are secreted into the extracellular medium. Overexpression of APP can accelerate Aβ secretion, which can form insoluble amyloid aggregates in the presence of amyloidotrophic factors, contributing to the development of Alzheimer disease.

Aβ has been found to produce hydrogen peroxide through metal ion reduction. As a result, Aβ toxicity in neuronal populations is believed to have a direct link to oxidative stress. Protein oxidative damage and DNA oxidation also have been observed in Alzheimer disease (45). Illustrations of oxidative stress in Alzheimer disease have gained further support through transgenic mice studies that reveal a direct correlation between oxidative stress and Aβ deposition (72). During the course of Alzheimer disease, neurotoxicity of Aβ may be the result of a progressive generation of oxygen free radicals and increased activity of cellular lipid peroxidation (53). Given the link between Aβ deposition and oxidative stress, agents that modulate ROS may be potentially useful in the therapy of Alzheimer disease. As a result, some therapeutic strategies have attempted to offer protection with antioxidant administration (74).

Oxidative damage is considered to be one of the main pathological mechanisms for Parkinson disease. Parkinson disease is a movement disorder characterized by tremor, rigidity, and bradykinesia. The pathophysiological basis of the disease is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). Oxidative stress may account for a significant degeneration of neurons in the SNc. Iron, which is a catalyst for the formation of hydroxyl radicals, was initially demonstrated to be increased in the SNc region (19). Subsequent work has now identified several different ROS products in this same region during Parkinson disease, such as lipid peroxides (27), protein carbonyls (2), and common oxidative products of nucleic acid 8-hydroxyguanosine (87). Each of these ROS products, as well as a combination of these agents, may be produced through a variety of iron-dependent and -independent pathways that ultimately impair SNc function and lead to neuronal injury and death.

In addition to precipitating chronic neurodegenerative disorders, oxidative injury also has been associated with acute ischemic brain injury. Oxygen free radicals, such as NO, can be produced during initial ischemic injury (43), as well as during periods of reperfusion with the production of superoxide radicals (26). Ischemia in the brain leads to the calcium-dependent activation of phospholipase A_2, the cleavage of membrane phospholipids, and the release of arachidonic acid. Superoxide radicals are subsequently produced when arachidonic acid is metabolized through cyclooxygenase and lipoxygenase. Generation of the free radical NO also is considered to be one of the "triggers" for the subsequent induction of neuronal injury. Enhanced expression of the enzyme responsible for NO production, nitric oxide synthase (NOS), has been associated with both chronic neuronal and vascular degeneration.

Interestingly, each isoenzyme of NOS, such as neuronal NOS (NOS-I), endothelial NOS (NOS-III), and inducible NOS (NOS-II), may differentially modulate cellular survival. During some injury paradigms, the generation of NOS-II in astrocytes or macrophages (66) has been suggested to be detrimental to neighboring neurons. As a corollary, it has been demonstrated that absence of NOS-II activity significantly increases neuronal and vascular endothelial cell viability during anoxia (43). Further analyses have illustrated that direct inhibition of NO production in cell culture systems during anoxia is cytoprotective (18, 50).

Some experimental models have argued for the protective effects of NO in the central nervous system (7). In some cases. increased production of NO has been shown to decrease cellular injury (32). Although these results have been attributed to increased cerebral perfusion, other studies suggest that improved cerebral perfusion alone to the ischemic zone is insufficient to sustain neuronal survival (49). Direct endothelial production of NO may be an important factor because this has been linked to the preservation of the antiapoptotic protein Bcl-2 through the down-regulation of cytosolic mitogen-actived protein kinase phosphatase-3 (61). It is unclear why certain environmental conditions may predispose NO to function as a protectant rather than a toxin. These divergent observations may involve parameters such as the experimental model, external environmental conditions, duration of the insult, age of the neuronal or vascular system, and the resultant NO species that is generated (10).

INDUCTION OF PROGRAMMED CELL DEATH THROUGH OXIDATIVE STRESS

Clinical investigations have offered evidence that apoptosis, also known as programmed cell death (PCD), may lead to neuronal injury in neurodegenerative diseases, such as Alzheimer disease (44). Both neuronal and vascular PCD proceed through two distinct pathways that are functionally independent (14, 22, 48). Although DNA degradation may immediately alter cellular integrity (29), the exposure of membrane phosphatidylserine (PS) residues can lead to acute cellular inflammation (20) and microglial phagocytosis of viable neurons (14, 28, 30, 31). Exposure of membrane PS residues is believed to occur prior to a later phase of genomic DNA degradation and serves to identify injured cells for phagocytosis (22, 48). An additional role of membrane PS externalization in vascular endothelial cells is the activation of coagulation cascades. The externalization of PS in platelets or endothelial cells can promote the formation of a procoagulant surface (20).

Oxidative stress can lead to apoptotic injury in two important cell populations of the brain, namely, neurons and endothelial cells (ECs). In neuronal populations, several studies have shown that ROS can alter cellular DNA integrity and membrane PS exposure. For example, exposure of neurons to ROS, such as NO or hydrogen peroxide, results in nuclei condensation and DNA fragmentation (13, 25, 59, 77). Externalization of membrane PS residues in neurons occurs under

several conditions with ROS exposure, such as with anoxia (11), during NO exposure (14, 77), and with the administration of agents that result in the production of ROS, such as 6-hydroxydopamine (64).

In ECs, oxidative stress can yield PCD during a broad range of insults that range from hypoxia (3, 11) to chlorinated oxidants (79). ECs that are exposed to ROS suffer both DNA fragmentation and membrane PS exposure (8, 11). In particular, treatment with the quinone compound dimethoxy-1,4-naphthoquinone, which is considered a source of oxygen free radicals, can induce EC apoptosis (5). Exposure to other ROS, such as NO, precipitates both the externalization of membrane PS residues and the destruction of DNA (11, 13).

An important caveat to PCD in both neurons and ECs is that the initial stage of apoptotic death, namely, membrane PS residue exposure, has been shown to be reversible. During the induction of apoptosis, progressive externalization of membrane PS residues occurs that is independent of the loss of cellular membrane integrity. Current techniques now offer the ability to monitor the induction and change in PCD in individual living cells over a period of time (48). Investigations that use these techniques have garnered support for the premise that neuronal PCD is reversible during ROS exposure. For example, the application of trophic factors (35), metabotropic glutamate receptor agonists (42, 78), benzothiazole compounds (48), Bcl-2 expression (21), or nicotinamide (47) has been shown to either prevent or reverse PS membrane externalization.

It is conceivable that these cytoprotective agents maintain membrane PS asymmetry through the maintenance of random "flip-flop" of membrane phospholipids through the prevention of lipid destruction by ROS. Alternatively, these agents may prevent changes in cellular energy metabolism during ROS exposure. Translocation of PS residues is associated with energy depletion and oxidative stress. Normally restricted to the inner leaflets of the plasma membrane, PS appears on the exoplasmic leaflet as a result of reduced aminophospholipid translocase activity and activation of a calcium-dependent scramblase (4). Maintenance of PS on the inner leaflet of the cell membrane is through the activity of a 120-Da Mg^{2+}-dependent ATPase. This ATPase-dependent activity is rapidly lost when cells are involved in apoptosis. As a result, the inhibition of the ATP-dependent aminophospholipid translocase during ROS exposure plays a critical role in PS externalization (24).

EARLY INFLAMMATORY MICROGLIAL ACTIVATION DURING OXIDATIVE STRESS

Microglia are monocyte-derived immunocompetent cells that enter into the central nervous system during embryonic development and function similar to peripheral macrophages. During an insult to the brain, microglia are the cells that are initially responsible for host defense. Microglia activation and the phagocytic removal of apoptotic cells within the central nervous system play an important role during development, tissue homeostasis, and host defense. The phagocytic removal of injured cells and foreign microorganisms can be considered to be beneficial for the preservation of cellular physiological homeostasis.

Generally maintained in a quiescent state, microglia become activated during a variety of pathological insults. The postmortem analysis of brain tissue in neurodegenerative disorders has provided strong evidence that microglial activation is involved in the progression of these diseases. In Alzheimer disease patients, microglial cells colocalize with the perivascular deposits of Aβ. In addition, microglial activation has been observed to occur in concert with the evolution of amyloid plaques (67). In both Huntington disease and amyotrophic lateral sclerosis, significant microglial activation has been reported in regions of the nervous system that are specific for these disease entities (57, 70). Furthermore, during cerebral ischemia, activation of microglia parallels the induction of cellular apoptosis and correlates well with the severity of the ischemic insult (62).

It is important to note that in the attempt to maintain tissue homeostasis as well as host defense mechanisms, microglia may sometimes further injury mechanisms rather than contain the cellular insult. Studies with microglia stimulated by phorbol myristate acetate have demonstrated the release of superoxide radicals. Application of scavenger agents for ROS, such as superoxide dismutase or deferoxamine mesylate, in the presence of activated microglia can prevent cellular injury. These studies suggest that ROS generated by microglia can be responsible for cellular cytotoxicity (76). Activated microglia also lead to cellular damage through the generation of NO and associated ROS products (65). The generation of ROS by microglia during Aβ deposition suggests that microglia may play an important role in the pathogenesis of neurological disorders such as Alzheimer disease. The secretion of cytokines by microglia also may represent another source of cytotoxicity for this cell population. Microglia produce a variety of cytokines in response to toxic stimulation, such as interleukins and tumor necrosis factor (TNF). TNF-α production by microglia may be linked to neurodegeneration by increasing the sensitivity of neurons to free radical exposure. For example, Aβ-induced microglial secretion of TNF-α during Aβ deposition leads to the neuronal expression of inducible NOS, peroxynitrite production, and neuronal apoptosis (16).

Yet microglia may better serve the central nervous system through the phagocytosis of apoptotic cells. The translocation of cellular membrane PS from the inner cellular membrane to the outer surface is a necessary prerequisite for phagocytosis (22, 30, 48). In cells that are without injury, the phospholipids of the plasma membrane are distributed asymmetrically with the outer leaflet of the plasma membrane consisting primarily of choline-containing lipids, such as phosphatidylcholine and sphingomyelin, and the inner leaflets consisting of aminophospholipids that include phosphatidylethanolamine and PS. Active maintenance of membrane phospholipid asymmetry is universal in cell membranes, and the disruption of this process that leads to the externalization of membrane PS residues is the initial sign of impending apoptosis.

In conjunction with membrane PS externalization for the identification of cells that have entered the early stages of apoptosis is the expression of the phosphatidylserine receptor (PSR) on microglia. Recent reports have demonstrated that neurons exposed to free radical injury can lead to the induction of both microglial activation and microglial PSR expression. Treatment with an anti-PSR neutralizing antibody in microglia prevents this microglial activation (14, 30, 31). In addition, application of PS could directly result in micoglial activation

that was blocked by PSR neutralizing antibody (14, 30, 31), suggesting that both PS exposure in target cells and PSR expression in microglia are necessary for microglial recognition of apoptotic cells in the nervous system.

Recognition of cellular membrane PS by the PS-specific receptors on microglia may require cofactors, such as Gas6. The protein Gas6 binds to negatively charged phospholipids, such as membrane PS, through calcium-dependent lipid binding domains and may be necessary for membrane PS to dock with PSRs (56). In addition, microglia recognition of injured neurons through membrane PS-mediated mechanisms also may involve other agents, such as integrin and lectin (83).

RE-ENTRY INTO THE CELL CYCLE DURING OXIDATIVE STRESS

A re-entrance into the cell cycle in postmitotic neurons can lead to the induction of PCD. Accumulating evidence has suggested that cell-cycle deregulation can trigger the late phase of DNA degradation. In the central nervous system, postmitotic neurons are incapable of differentiation. Yet, during a toxic insult, these neurons retain the ability to enter into the cell cycle with the alteration of some cell-cycle proteins, such as cyclin, cyclin-dependent kinase (CDK), and the retinoblastoma gene product (pRb) (58).

The expression of mitotic cyclins and their associated kinases has been reported during periods of neurodegenerative disease (9). Several studies support the premise that loss of cell-cycle regulation during oxidative stress can result in neuronal apoptosis. Induction of oxidative stress, such as during the application of dopamine or hydrogen peroxide, leads to the expression of the cell cycle-related genes cyclin B and CDK5 with subsequent induction of neuronal apoptosis (68). During injury paradigms such as anoxia or NO exposure, phosphorylation of the cell-cycle protein, pRb, appears to promote PCD, whereas the enhancement of hypophosphorylated pRb expression prevents the induction of PCD (46). In ischemic *in vivo* models, loss of CDK inhibitors may occur, suggesting that induction of the cell cycle leads to ischemic PCD (33). Studies that have used the cell-cycle markers bromodeoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) have provided further evidence that cell-cycle induction in postmitotic neurons that are destined to enter PCD can lead to neuronal apoptosis (41). In some instances, cell-cycle induction during oxidative stress in postmitotic hippocampal neurons can occur in concert with both the early and late stages of neuronal apoptosis that involve cellular membrane PS externalization and genomic DNA fragmentation, respectively (Fig. 1).

AKT AS A KEY REGULATORY ELEMENT DURING OXIDATIVE STRESS

Although a variety of cellular pathways may determine the fate of a cell during oxidative stress, the ability to rescue cells prior to their commitment to succumb to PCD may rest on the identification of biological targets that can regulate "pro-apoptotic" mechanisms. One such target that may provide a therapeutic avenue to prevent cellular injury and inflammation is protein kinase B, also referred to as PKBα or Akt. The serine-threonine kinase Akt is closely linked to cell growth and survival and functions as a downstream target of phosphoinositide 3-kinase (PI 3-K). Following stimulation by agents such as trophic factors or cytokines, PI 3-K is recruited to the plasma membrane, phosphorylates glycerophospholipid phosphatidylinositol 4,5-bisphosphate, and yields phosphatidylinositol 3,4-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3). As a cytosolic protein, Akt translocates to the cell membrane as a result of its binding to PIP2 and PIP3 through their respective plecktrin homology domains, and subsequently becomes activated through phosphorylation by phosphoinositide-dependent kinase 1 (82).

Once phosphorylated, Akt modulates the activity of several substrates, such as Bad, caspase 9, IκB kinase α, the forkhead transcription factor (FHKRL1), and the glycogen synthase kinase-3β (GSK3β), that are intimately involved with PCD. The Bcl-2 family member Bad promotes apoptosis by binding to Bcl-x_L and masking the antiapoptotic function of Bcl-x_L. Phosphorylation of Bad by Akt leads to the binding of Bad with the cytosolic protein 14-3-3 to release Bcl-x_L and allow it to block apoptosis (40). Phosphorylation of FKHRL1 by Akt also leads to the association of FKHRL1 with the 14-3-3 protein. As a result, FKHRL1 cannot translocate to the nucleus for the induction of PCD (6). As overexpression of GSK-3β can lead to apoptosis, Akt results in the blockade of PCD by inhibiting GSK-3β expression (17). Akt also promotes IκB kinase activity that results in the degradation of IκB and the liberation of nuclear factor-κB (NF-κB) (60). Free NF-κB subsequently translocates to the nucleus and leads to the induction of inhibitor of apoptosis protein c-IAP1, c-IAP2, and X-chromosome-linked IAP (xIAP).

Given its central role in a number of cellular pathways that are tied to apoptosis, Akt can be viewed as an attractive therapeutic strategy to either prevent or reverse toxic insults during oxidative stress. Yet, to further this premise for Akt, it becomes essential to elucidate the cellular pathways modulated by Akt during cellular injury in the central nervous system. Increased expression of phosphorylated Akt can occur in a variety of conditions that result in oxidative stress, such as during excitotoxicity (34), free radical exposure (15, 52), hypoxia (11), and trauma (54).

More importantly, enhanced activity of Akt can confer resistance against neuronal injury and oxidative stress. There exists evidence that the Akt pathway may be directly relevant to several clinical neurodegenerative disorders. For example, recent work has revealed that a potential antiapoptotic function of APP can be mediated through the PI 3-K/Akt signaling pathway. This pathway has provided protection against neuronal apoptosis caused by the Aβ, a major constituent of plaques in Alzheimer disease (51). Protein kinase B also may block the generation of oxygen free radicals by 1-methyl-4-phenylpyridinium, a dopaminergic neurotoxin that can lead to a parkinsonian-like syndrome (63). To a similar degree, Akt can serve as a survival factor during cerebral ischemia. Increased phosphorylation of Akt has been observed over different time periods in brains following focal cerebral ischemia (23). Periods of ischemic preconditioning appear to generate the most significant increases with Akt activation (85).

Furthermore, Akt has been identified as a possible critical factor for the maintenance of membrane asymmetry. Akt con-

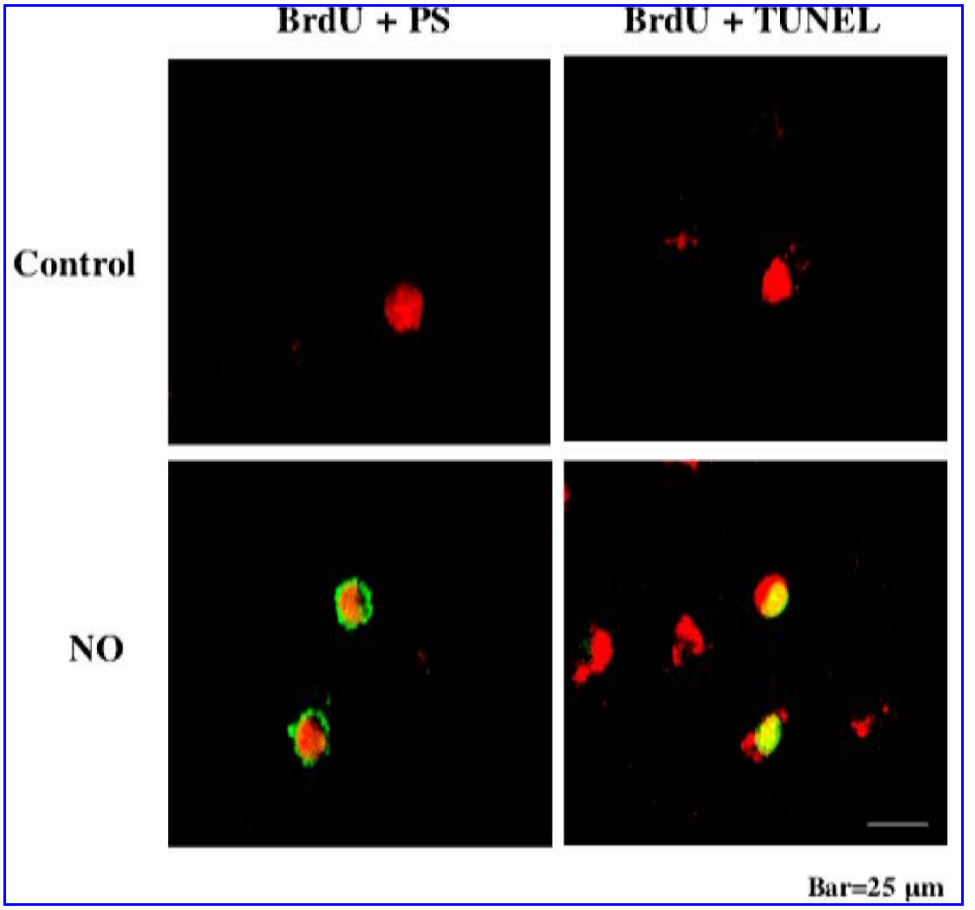

FIG. 1. Cell-cycle induction leads to membrane PS exposure and DNA fragmentation in postmitotic neurons during NO exposure. Dual fluorescence labeling for BrdU and PS (annexin V labeling) or BrdU and terminal deoxynucleotidyl transferase nick end labeling (TUNEL) (DNA fragmentation) in the same hippocampal neuronal cultures was performed 24 h following NO exposure (NOC-9, 300 μ*M*). Combined BrdU and PS labeling (red/green fluorescence, dual emission filter with 515–545 nm for PS and emission at 585–615 nm for BrdU) or BrdU and TUNEL labeling (red/yellow fluorescence, dual emission filter with 515–545 nm for TUNEL and emission at 585–615 nm for BrdU) was significantly increased in the same neurons exposed to NO. Control, untreated neurons not exposed to NO.

tains specific plecktrin homology domains that modulate the spatial regulation of actin assembly, suggesting a relationship between these domains of Akt and the coordination of cytoskeletal organization (37). More recent work has shown that Akt is a necessary component to modulate membrane PS externalization and protect cells from inflammatory injury and phagocytic removal (30, 31). There are several lines of evidence to support this premise. First, microglial activation and proliferation as assessed through PCNA expression, PSR expression, and BrdU uptake, occur during NO exposure (14). Second, application of an antibody to the PSR prevents microglial activation and proliferation, suggesting that membrane PS residue exposure is both necessary and sufficient to induce microglial activation and proliferation (14, 30, 31). Third, media taken from cells that overexpress active, phosphorylated Akt (myr-Akt) during either anoxia or free radical exposure show a significant reduction in the expression of PCNA, the expression of PSR, and the uptake of BrdU (30, 31). Taken together, this work illustrates that Akt can directly modulate microglial activation and proliferation through membrane PS exposure on cells, as well as possibly prevent the shedding of membrane PS residues that is known to occur during apoptosis (69).

Our knowledge of the underlying mechanisms that may determine the ability of Akt to protect against cellular mechanisms of neuronal apoptosis continues to evolve and will require further definition. Activation of endogenous Akt has been shown to provide a minimal level of cellular protection during several experimental paradigms that yield PCD during oxidative stress (15, 30, 31, 81). Overexpression of active Akt provides an additional degree of protection against both DNA fragmentation and cellular membrane PS exposure (30, 31) (Fig. 2). Although Akt can independently prevent oxidative stress in the nervous system, it may require modulation of additional cellular partners, such as tropic factors or cell-cycle proteins, to yield a broader degree of protection. For example, protection by vascular endothelial growth factor (38) and insulin-like growth factor-1 (52) has been closely tied to Akt activity. In addition, novel protection by erythropoietin in both neuronal and vascular cell populations has been demonstrated to rely on Akt (11, 12), whereas a feedback loop may be necessary between Akt and pRb activation (86).

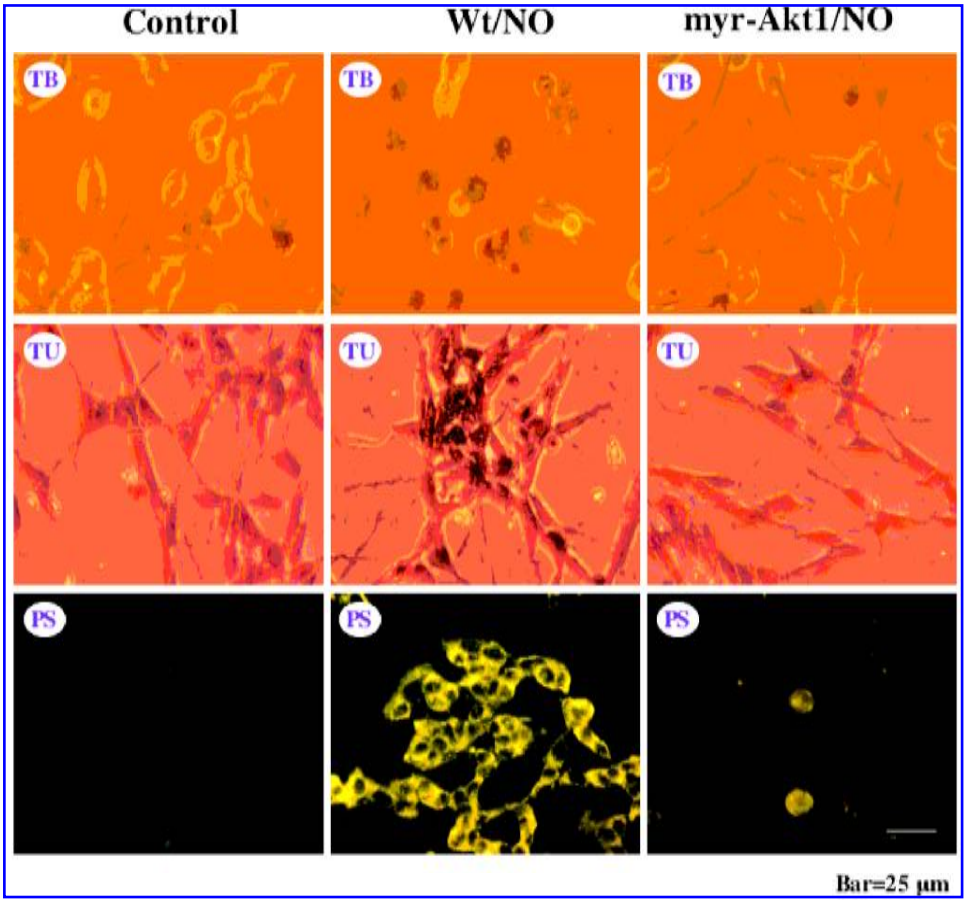

FIG. 2. Akt1 maintains cellular survival, genomic DNA integrity, and membrane PS asymmetry during NO exposure. Representative images illustrate cell survival with a trypan blue dye exclusion (TB), DNA fragmentation with TUNEL (TU), and PS exposure with annexin V phycoerythrin labeling (PS) in both wild-type (Wt) and myr-Akt1 (active form of Akt1) transfected SH-SY5Y (myr-Akt1) cells 24 h following exposure to a NO donor (NOC-9, 300 μ*M*). NO-induced cellular injury (cellular membrane integrity, DNA fragmentation, and membrane PS exposure) was evident in wild-type cells (Wt/NO), whereas there was no injury evident in Akt1-transfected cells (myr-Akt1/NO).

An intricate series of cellular pathways may be responsible for cytoprotection by Akt. Tolerance against oxidative stress by Akt may occur through the inactivation of its downstream components, such as Bad (14, 40) and FKHRL1 (6). Yet the ability of Akt to modulate specific cysteine protease activities may play a more encompassing role in the protection conferred by Akt. Both the maintenance of cellular integrity and cellular membrane asymmetry are dependent on cysteine protease activity. Specific cysteine proteases are associated with the independent apoptotic pathways of genomic DNA cleavage and cellular membrane PS exposure (13, 15, 75). Caspase 9 is activated through a process that involves the cytochrome *c*–Apaf-1 (apoptotic protease-activating factor-1) complex (13, 39). In addition, caspase 8 serves as an upstream initiator of executioner caspases, such as caspase 3, and also leads to the mitochondrial release of cytochrome *c* (73). Following caspase 8 and caspase 9 activation, genomic DNA degradation and membrane PS exposure can ensue through the activation of caspase 3 and caspase 1 (75). Akt can modulate DNA degradation through caspase 3 and prevent membrane PS exposure primarily through the inhibition of caspase 1-like activity and, to a lesser degree, through caspase 9- and 3-like activities (15, 30, 31). Caspase 1 is believed to play a significant role in the externalization of membrane PS residues in several cell systems through the digestion of cytoskeletal proteins, such as fodrin (11, 13, 48, 55). In addition, caspase 1 is believed to be responsible for the ability of Akt to regulate the activation and proliferation of microglia during oxidative stress (30, 31).

FUTURE DIRECTIONS

Oxidative stress is considered to be a principal mechanism in the pathogenesis of a variety of neurodegenerative disorders (Fig. 3). In the nervous system, apoptotic cell death proceeds through two distinct pathways that involve the early externalization of membrane PS residues and the late induction of DNA fragmentation. Early exposure of membrane PS is responsible for microglial activation and the phagocytic disposal of injured cells. In contrast, a later phase consisting of genomic DNA degradation appears more closely tied to an

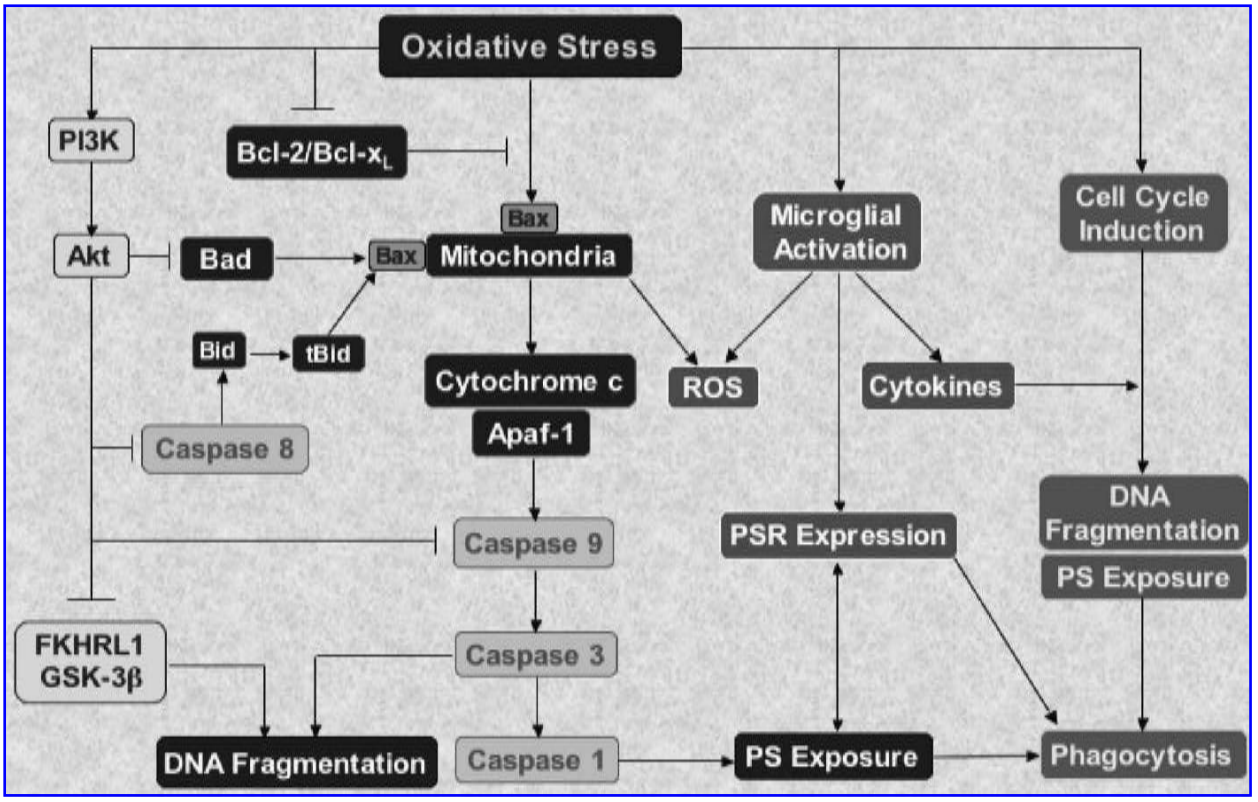

FIG. 3. Signaling pathways that mediate cellular injury during oxidative stress. Oxidative stress in the nervous system results in apoptotic cell death following exposure to ROS through two distinct pathways that involve the early externalization of membrane PS residues and the late induction of DNA fragmentation. Early exposure of membrane PS is responsible for microglial activation and the phagocytic disposal of injured cells. Genomic DNA degradation, a late phase of PCD, can be propagated through an ill-fated attempt to enter the cell cycle in postmitotic neurons. Central to the control of both extrinsic pathways of inflammation and intrinsic pathways of cellular integrity is the cytosolic protein Akt and its regulation of specific cysteine proteases. Protein kinase B (Akt) possesses the novel ability to maintain cellular integrity and prevent cellular removal through inhibition of microglial activation and proliferation. Akt prevents DNA degradation through a series of cellular pathways that involve caspase 1, caspase 3, caspase 8, and caspase 9. Additionally, maintenance of cellular membrane asymmetry that prevents PS externalization can be modulated through caspase 1 activation that protects against microglial activation with subsequent phagocytic destruction of cells.

ill-fated attempt to enter the cell cycle in postmitotic neurons. Central to the control of both extrinsic pathways of inflammation and intrinsic pathways of cellular integrity is the cytosolic protein Akt and its regulation of specific cysteine proteases. Although usually considered to be cytoprotective in nature, Akt represents a critical pathway for a host of cellular mechanisms that may promote or prevent a synergistic relationship between inflammatory cells and neurons in the nervous system. Only through the progressive understanding of the specific cellular and molecular mechanisms that determine cell survival during oxidative stress can we begin to harness the therapeutic potential of regulatory elements, such as Akt.

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ABBREVIATIONS

Aβ, β-amyloid; Apaf-1, apoptotic protease-activating factor-1; APP, amyloid precursor protein; BrdU, bromodeoxyuridine; CDK, cyclin-dependent kinase; EC, endothelial cell; FHKRL1, forkhead transcription factor; GSK-3β, glycogen synthase kinase-3β; IAP, inhibitor of apoptosis protein; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; PCD, programmed cell death; PCNA, proliferating cell nuclear antigen; PI 3-K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 3,4-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; pRb, retinoblastoma gene product; PS, phosphatidylserine; PSR, phosphatidylserine receptor; ROS, reactive oxygen species; SNc, substantia nigra pars compacta; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyltransferase nick end labeling.

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