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Comprehensive Invited Review

Neuronal Apoptosis in Neurodegeneration

MASAHIRO OKOUCHI,[1,3] OLEKSANDR EKSHYYAN,[2,3] MAGDALENA MARACINE,[2] and TAK YEE AW[2]

Reviewing Editors: Vittorio Calabrese, Maria Ciriolo, Jeffrey Keller, and Pamela Maher

[1]Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.
[2]Department of Molecular and Cellular Physiology, Louisiana University Health Sciences Center, Shreveport, Louisiana.
[3]MO and OE contributed equally to this work and are joint first authors.

ABSTRACT

Apoptosis mediates the precise and programmed natural death of neurons and is a physiologically important process in neurogenesis during maturation of the central nervous system. However, premature apoptosis and/or an aberration in apoptosis regulation is implicated in the pathogenesis of neurodegeneration, a multifaceted process that leads to various chronic disease states, such as Alzheimer's (AD), Parkinson's (PD), Huntington's (HD) diseases, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and diabetic encephalopathy. The current review focuses on two major areas (a) the fundamentals of apoptosis, which includes elements of the apoptotic machinery, apoptosis inducers, and emerging concepts in apoptosis research, and (b) apoptotic involvement in neurodegenerative disorders, neuroprotective treatment strategies/modalities, and the mechanisms of, and signaling in, neuronal apoptosis. Current and new experimental models for apoptosis research in neurodegenerative diseases are also discussed. *Antioxid. Redox Signal.* 9, 1059–1096.

I. INTRODUCTION

Apoptosis or programmed cell death is a highly organized and orchestrated form of cell death that is important in tissue homeostasis and is common to a variety of biological processes including normal organ involution, immune response, and embryogenesis. Cellular apoptosis is best characterized by morphological nuclear changes that are distinct from those elicited by necrotic death. In the central nervous system (CNS), neuronal apoptosis is a physiological process that is an integral part of neurogenesis, and aberrant apoptosis has been implicated in the pathogenesis of neurodegeneration. The recognition that loss of apoptotic control can underpin disease pathogenesis has spurred much research in the areas of apoptosis regulation and/or induction. A notable advancement in recent years is the emergence in our understanding the contribution of the interaction of cytochrome *c* with cardiolipin to its mitochondrial-to-cytoplasmic translocation. Our recent work implicates mitochondrial redox and DNA status as determinants of apoptotic susceptibility. Ample literature evidence links apoptosis to the neurodegenerative process. Much progress in the understanding of apoptotic involvement and signaling in neurodegenerative diseases was underpinned by the availability of cell systems as well as chemical- and genetic-based animal models. In addition, studies at the cellular, animal, and human levels on neuroprotective strategies that target attenuation of neuronal oxidative stress and apoptosis, as well as preservation of neuronal mitochondrial integrity, offer promise for future development of treatment modalities against neurodegenerative processes.

The spirit of the current review is not to provide an exhaustive and comprehensive coverage of the field, but rather to span from the fundamental concepts and mechanisms to the pathophysiological and health implications of apoptosis in neuronal cell death and neurodegeneration. The basic paradigms of apoptosis including the apoptotic machinery, its inducers, and emergent new concepts are discussed in Section II. It should be emphasized that the advancement of our present understanding of the fundamental concepts in apoptosis, *viz*, intracellular signaling, mechanism of regulation and cellular/molecular targets, results from the composite research efforts of numerous investigators using a variety of mammalian cell types. Some of the newer paradigms have yet to be validated in neuronal cells, and should provide exciting avenues for future research in the field of neuronal apoptosis and its relevance to neurodegeneration. The bulk of this review will be devoted to the coverage of the evidence for an apoptotic role in neuronal cell death and its implication for neurodegenerative diseases (Section III) and to neuroprotective strategies (Section IV).

II. APOPTOSIS AS A MECHANISM OF CELL KILLING

A. Overview of different forms of cell death

Classically, apoptosis and necrosis are considered to be distinct forms of cell death. Apoptosis is an active, highly regulated form of cell death characterized by cell shrinkage, chro-

matin condensation, DNA fragmentation, membrane blebbing, and the formation of apoptotic bodies that are rapidly phagocytosed by macrophages. Apoptosis is an energy-dependent process that requires ATP for protein synthesis and signal activation such as apoptosome formation and protein kinase-mediated phosphorylation reactions (82, 148). Indeed, an ATP threshold is required for a cell to undergo apoptosis. When the depletion of ATP is severe, apoptotic cell death is replaced by necrosis. Necrosis is typically a nonphysiological, unregulated pathological form of cell death characterized by chromatin clumping and swelling of intracellular organelles in the early stages, and disintegration of cell organelles and membranes in the later stages. Notably, during necrosis, the outer cellular membrane is disrupted and the intracellular components are released into the intercellular space, resulting in an inflammatory response. Moreover, caspase cascades are not activated and the morphological characteristics of apoptosis are absent (297).

Whereas necrosis is classically viewed as an accidental form of cell death, recent evidence indicates that certain cells can adopt a "programmed necrosis" or necroptosis phenotype upon FasL or TNFα stimulation (73). In this instance, the kinase activity of receptor interacting protein 1(RIP1), a protein that is recruited at the death inducing signaling complex (DISC) by death receptor activation, which normally is characteristic of apoptosis, was found to mediate necrotic cell death. The function of RIP1 in cell necrosis was confirmed in studies where the hyperactivation of poly (ADP-ribose) polymerase-1 (PARP-1) in mouse embryonic fibroblasts (MEFs) was mediated by RIP1, TNRF-associated factor 2 (TRAF2) and c-Jun N-terminal kinase (JNK) signaling. Activated JNK induced mitochondrial membrane potential ($\Delta\Psi$m) change and release of apoptosis inducing factor (AIF) that was associated with cell necrosis (403). It is noteworthy that these recent findings of overlapping players in the apoptotic and necrotic death pathways have blurred the classical distinction between the two forms of cell death.

Autophagy, as distinct from apoptosis and necrosis, is a caspase-independent form of cell death in which the cytoplasm is destroyed by lysosomal enzymes. Autophagy is an evolutionarily conserved pathway that involves catabolic degradation of large protein complexes or intracellular organelles by lysosomes (195) and thereby functions in the elimination of damaged organelles, and in cell remodeling during development and differentiation. Interestingly, autophagy was first described in yeast as a prosurvival mechanism because membrane lipids and proteins are recycled for mitochondrial ATP production when nutrients are limiting. During oxidative stress or starvation, autophagy can mediate cell death; for instance, pro-apoptotic stimuli result in autophagy of the Bax/Bak double knock out MEFs (218).

All three forms of cell death have been observed during neurodevelopment and/or neurodegeneration (214, 414). The focus of this review will be on neuronal apoptosis as an important player in neuronal death in the neurodegenerative process. It warrants mention, however, that much of the existing evidence for a relationship between neuronal apoptosis, oxidative stress, and neurodegeneration is circumstantial. Moreover, the conclusions drawn are based on classical methods of apoptosis detection that cannot unequivocally rule out contributions of other forms of cell death, such as "programmed necrosis," which we now know share similar death markers and/or characteristics with apoptosis (see above). Given the complexity of the neurodegenerative condition, there is no definitive evidence to date that neuronal apoptosis is the primary causal event in disease initiation or progression. Nevertheless, the collective early morphological observations and simple biochemical assays, and more recent studies using gene- and metabolic-based cell and animal models do provide good evidence linking neurodegeneration with significant death of neurons by an apoptotic process.

B. Apoptosis and components of the apoptotic machinery

Cellular apoptosis is a complex process that is triggered by extrinsic and intrinsic signals. The extrinsic (external) pathway involves the activation of a death receptor upon binding of its ligand, recruitment of specific proteins at the "death domain," and downstream signaling through a cascade of protein–protein interactions. The intrinsic pathway involves the mitochondria and the release of pro-apoptotic factors into the cytosol with subsequent activation of executioner caspases.

1. Death receptors. Death receptors belong to the tumor necrosis factor (TNF)/nerve growth factor receptor superfamily and are transmembrane proteins with three domains: an extracellular ligand-binding domain, a transmembrane domain, and a C-terminal domain. Death receptors are involved in transmitting the death signal from the cell surface to intracellular pathway upon substrate binding (72, 252). The best-characterized death receptors which function in apoptosis are Fas (CD95 or APO-1), TNF receptor 1(TNFR1), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) (death receptor 4, DR4), and TRAIL receptor 2 (TRAIL-R2) (death receptor 5, DR5) (11). A soluble Fas decoy receptor, DcR3, and three decoys for TRAIL receptors, TRAIL-R3 (decoy receptor 1, DcR1), TRAIL-R4 (decoy receptor 2, DcR2), and OPG (osteoprotegerin) have been characterized, but they are unable to trigger apoptosis because of the absence or truncation of the cytoplasmic death domain (10, 333).

The ligands that bind to the death receptors belong to the TNF superfamily of cytokines, which is comprised of TNF-α, Fas ligand (FasL), and TRAIL. The first step in receptor-mediated apoptosis is initiated by ligand binding to the death receptor, a process that causes receptor trimerization and recruitment of adaptor proteins at the cytosolic death domain (DD) of the receptor. Fas- and TRAIL-associated death domain (FADD/TRADD) proteins further bind pro-caspases 8 and/or 10 and form the DISC complex where the initiator caspases are activated. Depending on the downstream-mediated signaling mode, cells are divided into two categories. Type I cells exhibit efficient DISC formation and strong activation of caspase 8 that directly activates the downstream executioner caspases 3 and 7 (288). In type II cells, DISC formation is weak and therefore insufficient to induce direct caspase 8 catalyzed apoptosis *per se*; rather it initiates the cleavage of a pro-apoptotic protein, Bid, that in turn engages mitochondrial apoptotic signaling. Thus, the mitochondria function as "amplifiers" that induce the activation of executioner caspases (198). Samraj *et al*. (317) recently demonstrated that type II cells strongly depend on caspase 9 activation and the mitochondrial amplification loop to

mediate Fas-induced apoptosis. This finding provides evidence for the "two-pathway model" *viz*., the extrinsic death receptor, and the intrinsic mitochondrial pathways, of cell apoptosis mediated by death receptor (317).

The death receptor pathway has been best described in the immune system. Activated T cells and NK cells are the major cells expressing FasL, and mutations in Fas or FasL in humans lead to the complicated immune disorder, autoimmune lymphoprolipherative syndrome (145). The quantitative extent to which death receptors mediate neuronal apoptosis and their role in neurodegeneration is unclear. Normally, the Fas–FasL system is weakly expressed in the CNS, including glial cells and neurons, and is associated with the maintenance of immune suppression and prevention of an inflammatory response (302, for review, see Ref. 57). Fas presence in developing brains was involved in neurite remodeling; recent evidence demonstrates that Fas mediates neuronal branching through its death domain, without caspase activation (424). Under conditions of stroke and ALS, an increased expression of Fas or FasL could induce neuronal apoptosis and contribute to neurodegeneration (71, 235, 237, 300). Thus, it appears that the Fas system within the CNS has a dual function, namely, the participation in neurite branching during development and in neuronal apoptosis during the disease state (424). In other studies, immunochemical staining revealed the constitutive expression of TNFR1 and TNFR2 in human neuronal progenitors and microglial cells, and a predominance of TNFR1 in astrocytes (83, 94).

In pathological conditions, elevated TNFα production and TNFα receptors expression were shown to contribute to neuronal cell death (200, 386). Dorr (84) demonstrated the constitutive expression of apoptosis-induced (TRAIL-R1 and R2) and nonapoptotic-mediating (TRAIL-R3 and -R4) receptors in human brain that are localized on neurons, astrocytes, and oligodendrocytes; interestingly, constitutive expression of TRAIL was not detected. However, increased TRAIL production occurs in brain macrophages or T cells during neurodegeneration, which contributes to death receptor-mediated neuronal apoptosis (347). Thus, the involvement of two different CNS cell types, namely, parenchymal and inflammatory cells, in neuronal apoptosis constitute a unique death receptor system that differs from that of other organs (for review, see Ref. 140). A generalized schematic representation of death receptor-mediated signaling is presented in Fig. 1. Other proteins that bind to and modulate the death receptor apoptotic pathway have been described and are listed in Table 1.

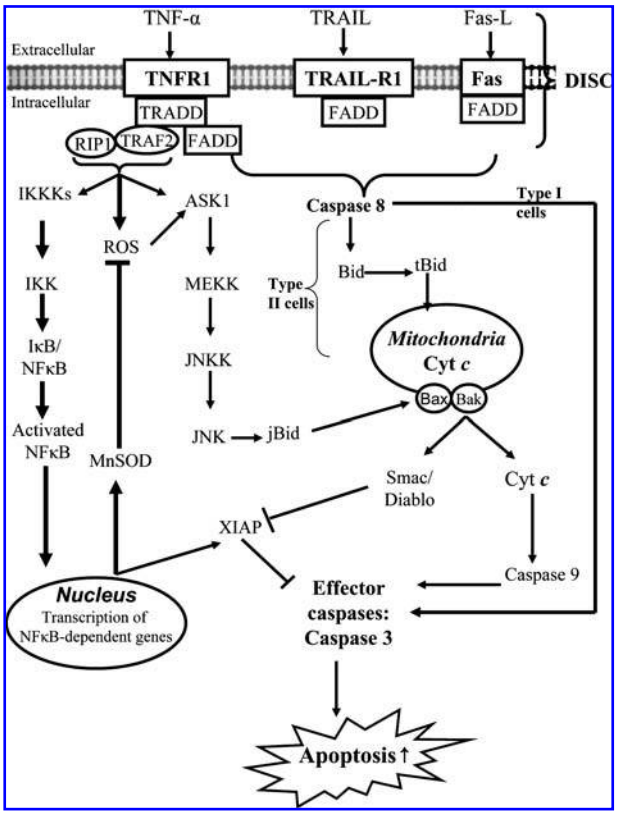

FIG. 1. Death receptor-mediated apoptosis and the link with the mitochondrial apoptotic pathway. The death receptor (extrinsic) pathway is activated upon ligand binding (such as TNFα), and mediates cell apoptosis through a cascade of protein–protein interactions that converge in the activation of executioner caspase 3. In type I cells, activation of caspase 8 can directly mediate caspase-3 activation and cell apoptotsis. In type II cells, caspase 8-induced cleavage of Bid mediates mitochondrial signaling that results in activation of caspase 3. In addition, an increase in ROS production after ligand binding induces JNK signaling that further contributes to mitochondrial apoptotic signaling through jBid. In parallel with initiation of apoptotic signaling, the engagement of death receptors can also activate NF-κB and anti-apoptotic signaling. Abbreviations are as in the text.

2. Mitochondrial pathway. It is well accepted that mitochondria are key players in the early induction and regulation of apoptotic cell death. Apoptotic stimuli such as DNA damage, ROS, or Fas signaling mediate mitochondrial cell death by a process that results in the release of small pro-apoptotic proteins that are normally located in the mitochondrial intermembrane space. Once in the cytosol, pro-apoptotic proteins like cytochrome *c*, second mitochondria-derived activator of caspases/direct IAP binding protein of low pI (Smac/Diablo), AIF, and endonuclease G (endoG), trigger caspase-dependent or -independent apoptotic death pathways (148). In the caspase-dependent mechanism, cytochrome *c* binds to an adaptor molecule, apoptotic protease-activating factor-1 (Apaf-1), to form the apoptosome where pro-caspase 9 is recruited and activated in the presence of ATP or dATP. Caspase 9 further cleaves and activates the effector caspases, procaspase 3 and/or 7, which process substrates like caspase-activated DNase (ICAD) or PARP, and leads to DNA fragmentation (310). In a caspase-independent manner, AIF translocates to the nucleus where it induces DNA fragmentation and chromatin condensation (123) while endoG induces internucleosomal DNA fragmentation (383). The pro-apoptotic molecules in mitochondrial-induced apoptosis are summarized in Fig. 2.

Several mechanisms have been proposed to explain the permeabilization of the mitochondrial membrane that leads to the release of small pro-apoptotic molecules. The first is the induction of the mitochondrial permeability transition (MPT), a process that involves the opening of a nonspecific mega pore, the permeability transition pore (PTP) (2). The opening of the PTP causes swelling of the mitochondrial matrix, which results

TABLE 1. PROTEINS THAT BIND AND/OR MODULATE THE DEATH RECEPTOR APOPTOTIC PATHWAY

Name	*Characteristics*	*Function*
1. *Fas-associated/modulatory proteins*		
cFLIP: cellular FLICE-inhibitory protein	Homologue of caspase 8 with no enzymatic activity	Inhibitor of caspase 8 at the DISC May signal NF-kB activation
Daxx: death domain-associated protein	Cytosolic protein	Regulates Fas-induced JNK pathway through ASK1 activation
FAP-1: Fas-associated phosphatase 1	Tyr-phosphatase	Inhibits Fas-mediated apoptosis
RIP1: receptor interacting protein 1	Ser/thr kinase	Mediates necrosis through kinase domain
FLASH: FLICE-associated huge protein	Cytosolic protein	Mediates Fas-induced apoptosis at the DISC
FAF-1: Fas-associated factor-1	Cytosolic protein	Enhances Fas-mediated apoptosis
FIST: Fas-interacting ser/thr-protein kinase	Ser/thr kinase	Modulates one or two signaling pathways of Fas
LFG: lifeguard	Cytosolic protein	Binds to Fas receptor and inhibits Fas-mediated death
Sentrin: small ubiquitin-related modifier-1	Cytosolic protein	Binds to Fas and prevents FADD recruitment
Ubc9: ubiquitin-conjugating enzyme	Ubiquitin-conjugating enzyme	Activates sentrin by ubiquitin modification at Gly-97
2. *TNFR1-associated/modulatory proteins*		
TRAF-2: TNF receptor-associated factor 2	Cytosolic protein	Recruits IKK complex from NF-kB pathway
RIP1: Receptor interacting protein 1	Ser/thr kinase	Stabilizes the TRAF2-IKK complex and its docking protein for RAIDD
RAIDD: RIP-associated ICH1/CED-3 homologous protein with DD	Cytosolic protein	Recruits caspase 2 after associated with RIP1
3. *TRAIL-R1 associated/modulatory proteins*		
IAPs: Inhibitors of apoptotic proteins	GTP-binding protein	Induces GTP-dependent action of caspase 8
DAP3: Death associated protein 3	Cytosolic protein	Inhibitor of TRAIL-induced caspase activation
cFLIP: Cellular FLICE-inhibitory protein	Homologue of caspase 8 with no enzymatic activity	Inhibitor of caspase 8 at the DISC
TRAF-2: TNF receptor-associated factor 2	Cytosolic protein	Recruits IKK complex from NF-kB pathway
RIP1: Receptor interacting protein 1	Ser/thr kinase	Stabilizes the TRAF2-IKK complex and its docking protein for RAIDD

in mitochondrial uncoupling, rupturing of the mitochondrial outer membrane, and release of pro-apoptotic proteins into the cytosol (408). Interestingly, recent findings suggest that this mechanism of MPT is mostly involved in cell necrosis and injury responses such as ischemia/reperfusion and localized mitochondrial Ca^{2+} overload (15, 139). The other important mechanism governing outer mitochondrial membrane permeabilization involves members of the Bcl-2 pro- and anti-apoptotic proteins. The Bcl-2 family is comprised of over 30 proteins and is divided into three groups according to their structure and activity. These are the anti-apoptotic proteins (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1/Bfl-1, NR-13, Boo/Diva/Bcl-2-L-10, and Bcl-B), the pro-apoptotic proteins (Bax, Bak, Bok/Mtd, and Bcl-Xs), and the BH3-only proteins (Bid, Bad, Noxa, Puma, Bmf, BimL/Bod, Bik/Nbk, Blk, Hrk/DP5, Bnip3, and Bnip3 L). The anti-apoptotic proteins have four Bcl-2 homology domains (BH 1-4), the pro-apoptotic proteins contain three BH domains, and the BH3-only proteins induce apoptosis by activating pro-apoptotic or by inhibiting anti-apoptotic proteins. Current evidence indicates that BH3-only proteins are activated by cytotoxic signals that promote activation of pro-apoptotic proteins of the Bax-like family, which induce their oligomerization. The oligomeric form of pro-apoptotic proteins has the ability to form pores and to penetrate different cellular membranes. The permeabilization of the outer mitochondrial membrane by pro-apoptotic Bax/Bak occurs through direct pore formation or through interaction with MPT pore components, in particular, with the voltage-dependent anion channel (VDAC) (7). The anti-apoptotic Bcl-2 proteins are the gatekeepers of mitochondrial membrane integrity and they prevent mitochondrial protein release by interacting with, and inhibiting, Bax/Bak and the BH3-only proteins. Indeed, the susceptibility of a cell to apoptosis is determined by the ratio between pro-and anti-apoptotic proteins. However, once the mitochondrial apoptotic signaling process has been initiated, a "point of no return" is reached and the anti-apoptotic Bcl-2 proteins no longer exert any effect (181).

Caspases are cysteine proteases that target aspartate residues of various substrates and are the main effectors of apoptosis. To date, 14 mammalian caspases have been described. Initiator caspases mediate the apoptotic signal elicited at the death receptor or at the mitochondria, and they possess either a death effector domain (DED) such as in caspases 8 and 10, or a caspase activation and recruiting domain (CARD) such as in caspases 2 and 9. Effector caspases like caspases 3, 6, and 7 are involved in the later stages of apoptosis. All caspases are synthesized in an inactive form as zymogens and are activated by apoptotic stimuli. Caspases 8 and 9 are the initiator caspases in the extrinsic and intrinsic apoptotic pathways, respectively, and are autocatalytically activated upon interaction with adaptor

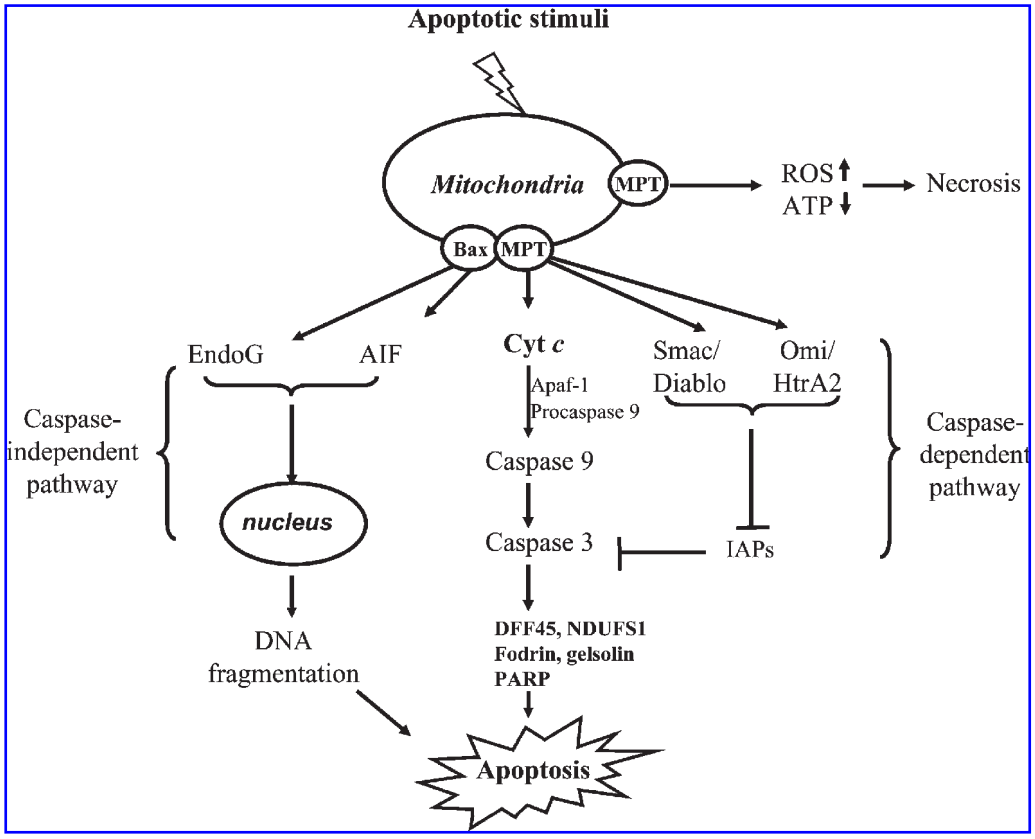

FIG. 2. Mitochondrial pathway of cell apoptosis. The mitochondrial (intrinsic) apoptotic pathway involves the release of pro-apoptotic factors located in the mitochondrial intermembrane space via mitochondrial permeability transition (MPT). Once in the cytosol, mitochondrial proteins, such as cytochrome *c,* Smac/Diablo, and Omi/HtrA2 mediate caspase-dependent, whereas EndoG and AIF induce caspase-independent apoptosis. Abbreviations are as in the text.

proteins (368). Effector caspases are activated by initiator caspases and they subsequently cleave various substrates to induce cell death. To date, >100 proteins have been identified as caspase substrates; these include mediators and regulators of apoptosis, namely, DNA fragmentation factor 45 kD subunit (DFF45/ICAD), 75 kD subunit of respiratory complex I (NDUFS1), structural proteins (fodrin and gelsolin), cellular DNA repair proteins [PARP, DNA-dependent protein kinase (DNA-PK)], and cell-cycle related proteins (Cdc27, Wee1, $p21^{CIP1}$, and $p27^{KIP1}$) (72). Inhibitors such as X-chromosomal inhibitor of apoptosis protein (XIAP), c-IAP1, c-IAP2, and surviving, regulate the intracellular activity of caspases. These inhibitors bind through their Baculovirus IAP repeat (BIR) domain to suppress the catalytic activity of caspases (77). During apoptosis, proteins like Smac/Diablo and Omi/HtrA2 are released from the mitochondria and act specifically on IAPs to block their effect (77, 357). Current evidence that support the involvement of mitochondrial apoptotic signaling in the progression and pathogenesis of various neurodegenerative conditions, including AD, PD and ALS, is presented in Section III, A.

C. Inducers of apoptosis

1. Reactive oxygen and nitrogen species. Reactive oxygen species (ROS) can be derived from exogenous sources or produced *in vivo*; these include the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2). ROS at low levels participate in cell signaling while higher ROS concentrations are deleterious due to the oxidation of proteins, lipids, and DNA. Additionally, persistent ROS production compromises the cellular antioxidant defense systems and results in oxidative stress and apoptosis (337). ROS can initiate apoptosis via the mitochondrial and death receptor pathways. In the former, ROS have been shown to induce loss of the $\Delta\Psi m$, release of mitochondrial pro-apoptotic proteins, and activation of caspase 3 (49). Our recent studies have demonstrated that cellular redox status plays an important role in mediating neuronal cell apoptosis. Specifically, we found that peroxide-induced apoptosis in undifferentiated PC12 cells was mediated by an early loss of the cellular glutathione–glutathione disulfide (GSH/GSSG) redox balance that preceded an increase in Bax expression, mitochondrial-to-cytosol cytochrome *c* translocation, and activation of caspase 3 (289–291). Apoptosis was ameliorated by the overexpression of mitochondrial superoxide dismutase, MnSOD (SOD2), and by pretreatment of cells with the antioxidant, *N*-acetyl cysteine (NAC) (289-291). Interestingly, we also found that PC12 apoptosis can be initiated by GSH/GSSG redox imbalance alone independently of ROS generation (291), suggesting that a loss of cellular redox homeostasis is downstream of ROS signaling in neuronal cell apoptosis.

ROS signaling has been shown to mediate cytokine-induced apoptosis. TNFα is a pro-inflammatory cytokine produced by macrophages and is the most studied cytokine in apoptosis and the pathophysiology of various diseases, including neurodegenerative disorders (145). Mechanistically, the binding of TNFα to its receptor activates the NF-κB and JNK signaling pathways believed to be mediated by ROS (Fig. 1). As first demonstrated in mouse fibrosarcoma cells, TNFα treatment disrupts mitochondrial electron transport and enhances ROS production (329). Recent studies by Han *et al.* (127) showed that modulation of the hepatocyte redox environment by ROS interfered with NF-κB signaling in TNF-induced apoptosis. Notably, cell apoptosis occurred within a certain redox window in which mild redox imbalance inhibited NF-κB activation, but not caspase activity. A role for ROS has also been implicated in death receptor-mediated apoptosis induced by apoptosis signal-regulating kinase 1 (ASK1), an ubquitiously expressed MAP kinase kinase kinase (MAPKKK), that activates JNK and p38 MAP kinase pathways. Intracellularly, endogenous ASK1 forms an inactive complex with inhibitory proteins like thioredoxin 1 (TRX1), in a complex named "signalosome" (256). Increased ROS oxidizes TRX1 and induces its dissociation from the inactive ASK1 complex (315).

The formation of "active signalosome" requires the recruitment of TRAF2 and 6 to ASK1 at the death receptor, which results in ASK1 activation (for review, see Ref. 97). Therefore, the ROS–TRX–ASK1 system functions as a sensitive redox switch whereby ROS acts as an apoptotic signal. Additionally, ROS can mediate ASK1 dissociation from protein complexes with glutaredoxin (GRX) and from binding with the docking protein 14-3-3 (112), or induce JNK activation by causing JNK dissociation from the complex formed with glutathione *S*-transferases (GSTs, isoform Π; 2). Prolonged JNK activation mediates the translocation of pro-apoptotic Bcl-2 proteins to the mitochondria, a process that triggers mitochondrial apoptotic signaling. For example, JNK can induce Bid cleavage to yield the specific death-promoting fragment, jBid, which translocates to the mitochondria and leads to the selective release of Smac/Diablo (75). The released protein neutralizes the action of IAP, resulting in caspase activation. This, in turn, cleaves more Bid (tBid) and promotes mitochondrial apoptotic signaling. Moreover, the phosphorylation of Bim by JNK enhances its pro-apoptotic activity through Bax-dependent mechanisms (192). JNK can further promote the dissociation of the 14-3-3/Bax complex through phosphorylation of 14-3-3, resulting in Bax translocation to the mitochondria (374). A recent study showed that Bax-mediated release of cytochrome *c* following JNK activation is a predetermining step in mitochondrial apoptotic signaling which is independent of JNK-mediated activation of Bid or Bim (273). The pathways of ASK1 activation and JNK-mediated apoptosis are summarized in Fig. 3.

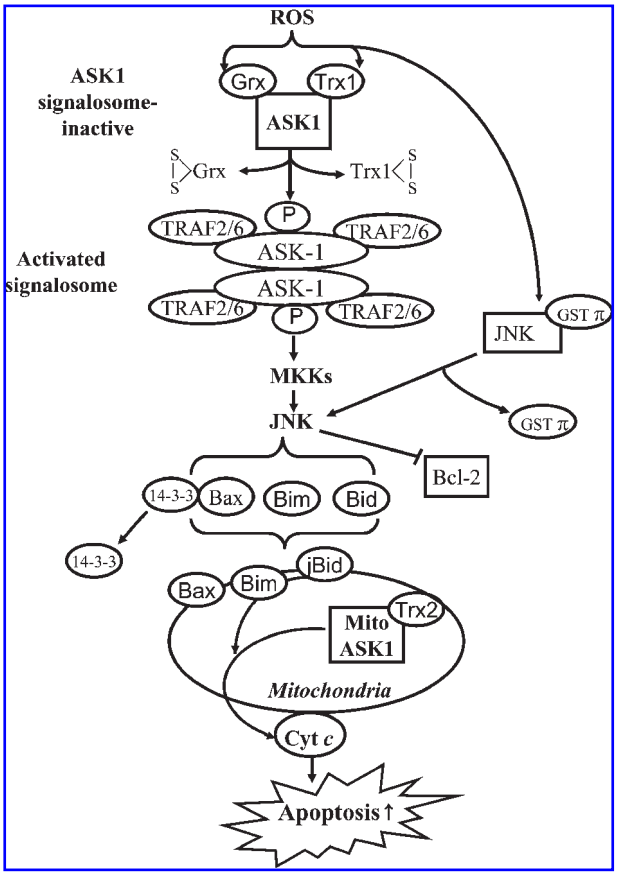

FIG. 3. ROS-induced activation of ASK and JNK signaling and cell apoptosis. An increase in cellular ROS can induce apoptosis through ASK1 and JNK signaling. Redox inactivation of the cytosolic ASK1 inhibitors, TRX1 and GRX, promotes ASK1 recruitment to the death receptor and forms "active signalosome." Subsequent activation of JNK signals translocation of pro-apoptotic Bcl-2 proteins to the mitochondria. ROS can also directly dissociate JNK from its complex with the GSTπ isoenzyme and initiates JNK signaling. In addition, ROS activates the mitochondrially localized ASK1 through dissociation from its inhibitor, TRX2. All these events converge in the initiation of mitochondrial apoptotic signaling that results in cell apoptosis. Abbreviations are as in the text.

Nitric oxide (NO) is produced from L-arginine by a family of NO synthase isoenzymes (NOS). Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutively expressed in tissues, and the low levels of NO produced functions in normal cell physiology. In contrast, iNOS, an inducible form of the enzyme, produces high levels of NO that are often detrimental to cellular components and can induce cell apoptosis (for review, see Ref. 270). NO-induced apoptosis is cell type dependent; whereas NO mediates apoptosis in macrophages, thymocytes, and neurons, it prevents apoptosis in B cells, splenocytes, hepatocytes, and ovarian follicles (182). Furthermore, in certain cell lines, such as human lymphoblasts, a threshold of NO concentration and cumulative cellular effects is necessary for the cell to undergo apoptosis (196). NO-induced apoptosis is associated with mitochondrial apoptotic signaling namely, expression of pro-apoptotic Bax (287), and activation of caspases 3 and 7 (250, 387). Interestingly, in neuroblastoma and HL-60 cells, NO can trigger nonmitochondrial signaling as evidenced by an early activation of caspases 8 or 2 (405).

The reaction of NO with metals, O_2, or O_2^-, produces reactive nitrogen species (RNS) like peroxynitrite ($ONOO^-$), nitrosonium (NO^+), dinitrogen trioxide (N_2O_3) that interact with cellular macromolecules inducing lipid oxidation, protein modification, or DNA damage. NO and related species can directly *S*-nitrosylate (S-NO), oxidize (RS-SR), or nitrate (R-NO_2) pro-

teins (167). *S*-Nitrosylation, which involves the attachment of an NO to a thiol (R-SH), is a reversible mechanism that controls protein activity in cell signaling and gene regulation (29). However, the production of high levels of nitrosylated proteins can induce nitrosative stress with deleterious consequences for the cell, namely, loss of protein function, decrease in cellular redox, or irreversible oxidative modification that has been linked to inhibition of cell growth and to apoptosis. Recent evidence implicates a role for RNS and NO-induced nitrosative stress in mitochondrial dysfunction and neuronal apoptosis (38, 270, 282). Specific discussion of the mechanism of NO-induced neuronal apoptosis is presented in Section III.A.

2. DNA damage. Cellular DNA damage induces a cascade of events that results in cell cycle arrest and DNA repair, or in cell apoptosis if the damage is extensive (16). Initiation of apoptotic signaling usually involves phosphatidylinositol 3-kinase (PI3K)-like kinases, such as ataxia-telangiectasia-mutated (ATM), ataxia-telangiectasia Rad3-related (ATR), and DNA-PKs. These kinases activate downstream checkpoint kinases like ChK1 and ChK2 that phosphorylate the transcription factor p53 and its inhibitor, the human double minute 2 (HDM2) protein, resulting in p53 activation, an important element of the intrinsic apoptotic pathway. Activated p53 then initiates mitochondrial apoptotic signaling through transcriptional upregulation of mitochondrial permeability proteins like PUMA, NOXA, Bax, and Bid, and downregulation of Bcl-2 and Bcl-xL (146). Additionally, p53 translocates to the mitochondria and directly induces outer membrane permeabilization and cytochrome *c* release.

A connection between cell cycle arrest and DNA damage-induced apoptosis has been recently described, which highlights the dual role played by the BH3-only pro-apoptotic protein, Bid. In MEFs treated with DNA-damaging agents, Bid transfered to the nucleus and was phosphorylated by ATM. The phosphorylated protein maintained genomic integrity and mediated cell cycle arrest in the S-phase, and thereby functions in a pro-survival role. Bid also participates in apoptosis; truncated Bid migrates to the mitochondria and induces Bax/Bad-mediated cytochrome *c* release. Thus, Bid serves as a mediator between cell death and cell survival in DNA damage signaling pathways (156, 421).

Recent studies propose a mechanism of DNA damage-induced apoptosis that involves caspase 2 (for review, see Ref. 379). Pro-caspase 2 activation is initiated by the spontaneous recruitment of the inactive zymogen to a protein complex containing RIP associated ICH1/CED3 homologous protein with DD (RAIDD) and a death domain, p53-inducible protein with DD (PIDD), called PIDDosome (379). Active caspase 2 has a unique property that can directly mediate mitochondrial membrane permeabilization and elicit the release of pro-apoptotic proteins into the cytosol or induce Bid cleavage and initiate Bax/Bad-mediated mitochondrial apoptotic signaling (276). A direct connection between caspase 2 activation and a cell's commitment to apoptosis has been demonstrated in several cell types; in HCT116, a human colon carcinoma cell line, the process is associated with the presence of p53-dependent protein PIDD (381). A schematic representation of DNA damage-induced apoptosis is illustrated in Fig. 4. As distinct from apoptosis, mitotic catastrophe that occurs during mitosis is a recently described new form of cell death that involves caspase 2 activation in response to DNA damage (for review, see Ref. 47). Whether such a mechanism is operative in neuronal cell death is unknown.

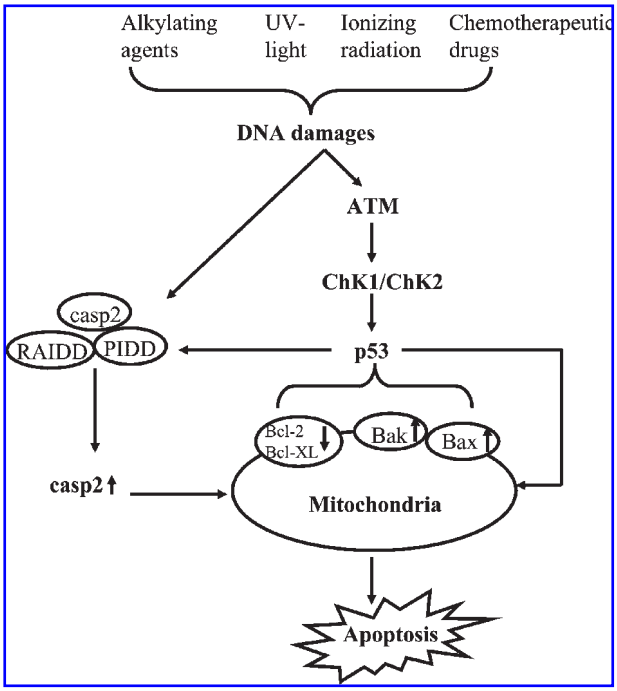

FIG. 4. DNA damage-induced apoptosis. Exposure of cells to UV-light, ionizing radiation or alkylating and chemotherapeutic agents induces DNA double-strand breaks or formation of bulky DNA adducts. If not repaired, these lesions result in the activation of p53 or caspase 2, which mediate cell apoptosis through mitochondrial apoptotic signaling.

D. Emerging concepts in apoptosis research

1. Cytochrome c/cardiolipin interaction as a mechanism of apoptosis initiation. It is well recognized that the activation of mitochondrial apoptotic signaling results in the release of pro-apoptotic proteins into the cytosol. Of these, cytochrome *c* is the most well studied (Section II.B and Fig. 2). Cytochrome *c* release from the mitochondria is a critical and early event in the mechanism of apoptosis. Within the cytosol, cytochrome *c* forms the apoptosome with Apaf-1 and pro-caspase 9 resulting in caspase 3 activation. Despite its centrality in apoptosis initiation, the mechanism(s) of mitochondrial cytochrome *c* release remains elusive. Much of the focus has been on cytochrome *c* release via mitochondrial permeabilization and its regulation by members of the Bcl-2 family. Recently, new insights have emerged on the underlying mechanism for cytochrome *c* mobilization from the mitochondrial inner membrane, which is prerequisite for its release. Studies have shown that cytochrome *c* can function as a mitochondrial electron carrier under physiological conditions and as a specific peroxidase during apoptosis (154). A large fraction of cytochrome *c* is loosely bound (85%) and readily detached by ionic salt; this fraction participates in electron transport and su-

peroxide scavenging. The remainder (15%) is tightly bound to cardiolipin, a phospholipid unique to the mitochondrial inner membrane. The cytochrome *c*–cardiolipin association is specific and stoichiometric and involves electrostatic interactions at the A site of cytochrome *c* and hydrophobic interactions and hydrogen bonding at its C-site (375). The cardiolipin-bound cytochrome *c* exhibits peroxidase activity that eliminates mitochondrial derived H_2O_2 and is important in apoptosis (52). Disruption of mitochondrial electron transport from apoptotic signals enhances $O_2^{\cdot -}$ and H_2O_2 generation, which promotes the peroxidase activity of cardiolipin-bound cytochrome *c*. This induces cardiolipin peroxidation, and results in cytochrome *c* dissociation.

It has been proposed that it is the breach of these cardiolipin-cytochrome *c* interactions that provides the mechanism for cytochrome *c* to leave the mitochondria. Specifically, oxidized cardiolipin migrates from the inner to the outer mitochondrial membrane, a process that is essential for cytochrome *c* release and tBid and Bax signaling (185). In this regard, cardiolipin-bound cytochrome *c* could be viewed as a mitochondrial oxidative stress sensor and redox regulator of apoptosis. This model of cytochrome *c*/cardiolipin interaction is in agreement with the proposed "two step" mechanism of cytochrome *c* release from the mitochondria during apoptosis (for review, 111). In the first step, cytochrome *c* is detached from cardiolipin at the inner membrane upon oxidation of the phospholipid. The second step involves cytochrome *c* release into the cytosol through Bax/Bak outer membrane permeabilization (268). Other studies demonstrated that the activation of cytochrome *c* to a peroxidase could also occur in the cytosol through interaction with phosphatidylserine (142). Oxidized phosphotidylserine is externalized on the plasma membrane and serves as a recognition signal for macrophages in phagocytosis of the apoptotic cell (89).

2. Mitochondrial ROS and GSH redox in oxidative DNA damage and apoptosis initiation. *Mitochondrial ROS and GSH redox and apoptosis.* ROS-mediated mitochondrial failure is a key event in oxidant-induced cell apoptosis, and impaired mitochondrial electron transport as occurs with oxidative challenge, can itself enhance ROS production. The resulting exaggerated mitochondrial ROS stress is expected to impact the oxidation of cardiolipin, its interaction with cytochrome *c* and the promotion of cytochrome *c* loss from the mitochondria (see above). The contribution of mitochondrial redox to apoptosis initiation remains to be defined despite a link between cellular GSH and cell apoptosis. The mitochondrial redox compartment, as distinct from the cytosolic compartment, maintains mitochondrial integrity. Thus, the depletion of matrix GSH, regardless of the cytosolic GSH status, is expected to be a key event in sensitization of cells to oxidant-induced apoptosis.

Early evidence suggests that mitochondrial GSH (mtGSH) influences apoptotic signaling through mitochondrial membrane permeabilization (93). Studies by Zamzami *et al.* using diamide, a thiol oxidant, demonstrated that pore function can be modulated by thiol oxidation in that functional divalent thiol-reactive agents induce formation of disulfide cross links which block Bcl-2 function in preventing permeability transition (415). Subsequent studies by the same groups showed that diamide, dithiodipyridine, and bis-maleimidmido-hexane induce the specific oxidation of Cys^{56}, a critical cysteine residue in the adenine nucleotide translocator (ANT), a component protein within the permeability transition pore complex (66), which leads to a membrane permeability response. Thiol oxidation was observed in purified ANT, in isolated mitochondria, and in intact cells. Moreover, recombinant Bcl-2 failed to prevent the thiol modification of ANT (66). Collectively, these results indicate that covalent modification of ANT by thiol cross-linking can induce permeability transition that is independent of Bcl-2 control. Using the thiol oxidizing agents, diamide and phenylarsine oxide, McStay *et al.* similarly demonstrated a role for critical thiol groups on the matrix surface of ANT in the mechanism of mitochondrial permeability transition (241). While these findings indicate that thiol oxidation *per se* can regulate pore function, it remains to be determined whether this mechanism operates under pathophysiologically relevant redox status conditions, such as increased mtGSSG, as would occur during oxidative challenge and mitochondrial failure.

a. Mitochondrial oxidative DNA damage and apoptosis. In recent years, an increased interest in mitochondrial DNA (mtDNA) damage has stemmed from the finding that mtDNA damage induces cell apoptosis (269). The mitochondrial genome is vulnerable to oxidative stress because of its open circular structure, lack of protection by histones, and its close proximity to the mitochondrial electron chain. A 3- to 10-fold greater damage to mtDNA than nuclear DNA has been measured during oxidative stress in human fibroblasts (316). The mitochondrial genome encodes several hydrophobic components of the respiratory chain, and mtDNA damage will decrease gene expression of key respiratory proteins (17). The consequent disruption in electron flow enhances ROS generation, which creates a vicious cycle of mitochondrial dysfunction that includes decreased ATP production and loss of $\Delta\Psi m$ that culminates in cell apoptosis (113). Unlike the nucleus, the mitochondria lack the sophisticated enzymatic machinery to repair bulky DNA damage but do possess an efficient base excision repair (BER) system comprised of DNA glycosylases and AP endonucleases, to remove oxidized bases like 8-oxodG (8-oxodeoxyguanosine) and apurinic/apyrimidinic (AP) sites. Accordingly, a decreased mitochondrial BER pathway correlates with mitochondrial initiated apoptosis. Dobson *et al.* (80) first demonstrated that enhanced mtDNA repair following oxidative stress induced by menadione is associated with cell survival. The same investigators also found that HA1 Chinese hamster fibroblasts adapted to increasing ROS concentrations, exhibiting higher levels of AP endonuclease 1 and repair rates, and consequently lower oxidative mtDNA damage after oxidant exposure (119).

We recently proposed a novel hypothesis that mtGSH redox is a determinant of mitochondrial genomic integrity and together, they control apoptotic initiation and cell susceptibility during oxidative challenge, a concept that has hitherto not been explored (231). Our initial study, in an intestinal cell line, showed that menadione-induced damage to mtDNA was associated with significant cell apoptosis. Both events were attenuated by NAC pretreatment, thus providing the first evidence that mtDNA susceptibility and cell demise due to oxidative challenge is redox sensitive. Importantly, the attenuation of oxidative mtDNA damage by NAC restored the cell's capacity for

mitochondrial ATP production and the maintenance of $\Delta\Psi m$, consistent with preservation of mitochondrial functional integrity (231). Our collective preliminary results may have uncovered a novel role for mtGSH redox in the preservation of mitochondrial genomic and functional integrity and of cell survival. Whether this mode of apoptosis regulation by mtGSH redox and mtDNA is a generalized paradigm in all cell types, including neuronal cells, is unresolved and awaits further experimentation. Possible involvement of oxidative mtDNA damage in neurodegenerative processes is broadly suggested by the observation that oxidant-mediated mutations in the subunits of complex I of mitochondrial electron transport are involved in Alzheimer's disease, Parkinson's disease, and in Leber's hereditary optic neuropathy (266).

III. APOPTOSIS IN NEURODEGENERATIVE DISORDERS

A. Neuronal death and apoptosis: general considerations

1. Apoptosis in normal neurodevelopment. During the development of the nervous system, an excessive number of neurons is produced. This massive overproduction of neurons is followed by a programmed demise of roughly one-half of the originally produced cells. The precisely controlled process is referred to as naturally occurring neuronal death which is a highly conserved cellular mechanism in diverse organisms, ranging from invertebrate species such as the nematode, *Caenorhabditis elegans,* and insects, to nearly all of the studied vertebrate species (243). Natural neuronal death is believed to mold the nervous system's cellular structure and function. Neuronal cells are eliminated by two big waves of programmed cell death, namely, the early death of proliferating precursors and young postmitotic neuroblasts, and the late death of postmitotic neurons. While the mechanism of the selective late death of postmitotic neurons is well explained by the competition among neurons for limiting amount of target-derived trophic factors (the neurotrophic theory), the regulation of the early wave of natural neuronal death is not well understood. There are suggestions that it is linked to cell cycle regulation (for review, see Ref. 24) and the removal of cells with irreparable DNA damage (96), or is regulated by factors such as bone morphogenetic proteins, Wnts, fibroblast growth factors, and Sonic Hedgehog (for review, see Ref. 411).

A growing body of evidence shows that the apoptotic death cascade mediates both waves of naturally occurring neuronal death. While *in vivo* studies are scanty, they provide evidence of apoptotic processes in the developing brain of different mammalian species. A high number of dying neurons with characteristic apoptotic morphology (pyknotic cells) has been detected in the developing postnatal rat cerebellum (183). In addition, DNA fragmentation was detected in the postnatal mouse (398), rat (183), rabbit (213), and human cerebellum (215), and during prenatal development of human cerebral cortex (389). Naturally occurring neuronal death in the rat brain is accompanied by a decreased Bcl-2 to Bax ratio with a concomitant increase in the expression of active caspase 3 (226). Studies in mice defective in cytochrome *c*-mediated pathway support an essential role of the apoptotic machinery in brain development (184, 412). Additional evidence comes from studies in primary cultures, cell lines, and transgenic animals as proper balance of pro- and anti-apoptotic factors was found to be crucial for the normal development of the nervous system. For instance, deficiency in pro-apoptotic proteins or overexpression of anti-apoptotic proteins leads to a variety of brain abnormalities, such as nervous system hypertrophy, aberrant structural organization in different parts of the brain, and reduced susceptibility to certain apoptotic stimuli. On the other hand, deficiency in anti-apoptotic proteins leads to decreased neuronal survival or exacerbation of neuronal apoptosis (214).

2. Trophic factors, ROS, and NO in neurophysiology, pathophysiology, and apoptosis. In agreement with the widely accepted neurotrophic theory of naturally occurring neuronal apoptosis, neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3, and neurotrophin 4/5, act via the tyrosine kinase growth factor receptors. They stimulate the prosurvival Ras/PI3K/protein kinase B (Akt) pathway, which inhibits the expression of pro-apoptotic proteins, but enhances the expression of antioxidant enzymes (50, 150, 159, 190). Other evidence suggests that the JNK/cJun signaling pathway plays an important role in both naturally occurring and pathogenic neuronal apoptosis (126, 208) (Fig. 5). A shortage of neurotrophic factors or increased oxidative stress in neuronal cells activates the JNK/c-Jun pathway in many systems. It is notable that JNKs have a wide range of action including neuronal differentiation, neurite outgrowth, and regeneration (88, 209, 401). These different actions of JNKs are in part due to their isoform-specific activation; to date, three JNK genes (JNK1-3) have been identified to generate 10 isoforms by differential splicing (21).

The high metabolic rate in neuronal cells is associated with an elevated basal cellular ROS production. Under physiological conditions, ROS flux is quenched by the abundant antioxidant systems in neuronal cells; a deficit of antioxidant defenses and an ROS overload leads to oxidative stress. Thus, a decline in different antioxidant systems such as occurs in aging, is a risk factor for the development of certain neurodegenerative disorders; for instance, a decrease in tissue GSH in the substantia nigra (SN) has been implicated in the pathogenesis of PD (336). Importantly, ROS act as signal molecules. The addition of NGF transiently increases intracellular ROS, and induces neuronal (PC12) differentiation that is prevented by *N*-acetylcysteine (NAC) or catalase (356). Similarly, neuregulins (NRG)-induced differentiation of PC12 cells is prevented by NAC pretreatment (113). Interestingly, the requirement for ROS during embryonic rat cortical cell differentiation is higher than in progenitor cells (373).

In addition to the importance of ROS, there is much evidence in the literature that implicates NO involvement in normal brain biology and pathobiology. Under physiological conditions, eNOS-derived NO plays a role in controlling cerebral blood flow, whereas nNOS-derived NO is important as a neurotransmitter that functions in synaptic plasticity, memory formation, or modulation of neuroendocrine activity (for review, see Ref. 38). Under pathological conditions, iNOS-derived NO production increases in glial cells, vascular cells, and infiltrating

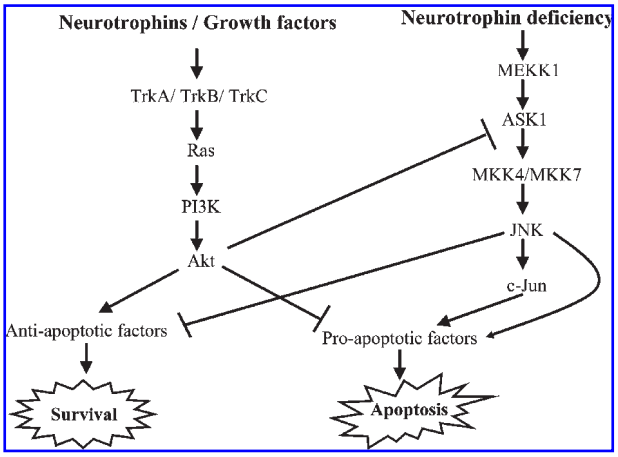

FIG. 5. Relationship between JNK/c-Jun signaling and neuronal apoptosis: modulation by prosurvival actions of neurotrophins and growth factors. Neurotrophins and growth factors acting via tyrosine kinase growth factor receptors promote cell survival by activating the prosurvival ras/PI3K/Akt pathway. This results in inhibition of pro-apoptotic proteins and increased expression of antioxidant enzymes. Neurotrophin deficiency inhibits the PI3K/AKT pathway and increases ROS production, followed by ASK1/JNK activation and c-Jun phosphorylation. c-Jun induces the expression of pro-apoptotic proteins. In addition, activated JNK can further directly activate certain pro-apoptotic BH3-only proteins and inhibit anti-apoptotic proteins through their phosphorylation.

phagocytes (121). The increased formation of $ONOO^-$ from $NO\text{-}O_2^{\cdot -}$ interaction has been implicated in cell death associated with neurodegeneration (211). Increased $ONOO^-$ can induce nitration of cysteine, methionine, or tyrosine residues of targeted proteins, resulting in conformational changes and loss of protein function; it has further been shown to initiate both apoptotic and necrotic death, depending on the dose and length of exposure (387). A growing body of evidence indicates that mitochondria are major targets of NO-induced nitrosative stress in neurological disorders. In this regard, NO irreversibly binds the components of the mitochondrial electron transport chain, resulting in impaired ATP production, loss of $\Delta\Psi m$, and release of pro-apoptotic proteins into the cytosol (350, for review see Ref. 270). In chromaffin cells, NO induces cytochrome *c* release and caspase 3 activation (387). In animal models, NO has been shown to mediate adult motor neuron apoptosis by Fas death receptor signaling and caspase 8 activation triggered by $ONOO^-$ induced oxidative DNA damage (235). It should be noted that significant DNA damage initiates NO/PARP signaling, induces severe ATP depletion, and elicits cell necrosis (292).

An interesting NO-dependent mechanism of neuronal death associated with late-onset of AD and PD, is the activation of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)/Siah1 cascade (for review, see Ref. 128). *S*-Nitrosylation of cytosolic GAPDH by NO facilitates the binding of Siah1, an E3 ubiquitin ligase, which when transported into the nucleus initiates the proteasome-dependent degradation of Siah1 substrates that cause apoptotic cell death (128). In Huntington pathology, the involvement of the NO/GAPDH/Siah1 cascade in neuronal death is linked to the translocation of mutant Huntingtin protein by the GAPDH/Siah1 complex (13, 200, 362). The accumulation of oxidized and misfolded proteins in neurodegenerative disease can lead to endoplasmic reticulum (ER) dysfunction that ultimately triggers the unfolded protein response (UPR) and initiates apoptosis through caspase 12 activation or cross-talk with the mitochondria (277). Since humans do not express a functional caspase 12, ER-induced neuronal apoptosis likely occurs through mitochondrial death signaling. The upregulation of PDI (protein-disulfide isomerase) represents an adaptive response to protect neuronal cells from UPR. A recent study by Uehara *et al.* (378) showed that *S*-nitrosylation of PDI in brains from patients suffering from PD and AD is associated with the loss of enzyme activity and neuronal apoptosis through activation of the UPR signaling pathway. Thus, NO-induced nitrosylation of PDI represents a link between ER stress and neurodegeneration.

Anti-apoptotic effects of NO have been described. Increasing evidence supports the hypothesis that NO regulation of gene expression confers protection against nitrosative stress in neuronal cells (41). One of the mechanisms that contribute to the inhibition of cell death by NO is the induction of the production of vitagens, including heat shock proteins (HSPs) such as HSP32, HSP60, HSP72, and heme oxygenase 1 (HO1), as well as the thioredoxin reductase system (38). Vitagens are genes that participate in so-called longevity assurance processes such as cellular maintenance and repair processes that are crucial for both survival and physical quality of life. Among these, the HSP system has been shown to confer cytoprotection in various human diseases including neurodegenerative disorders (41). HSPs bind to denaturated proteins and serve as chaperones to preserve their conformation. The HO-catalyzed production of bilirubin, the end product of heme degradation, has been shown to protect against $ONOO^-$ (91). In fact, recent evidence implicates the HO system as the main defensive mechanism in neurons against oxidative challenge (227). The expression of detoxification or antioxidant enzymes such as MnSOD, glutathione-*S*-transferase, NADPH: quinone reductase, as well as intracellular glutathione, can also be elevated after NO exposure, and confer neuroprotection (40). Thus, the induction of specific antioxidant genes in the CNS, particularly the heat shock response genes, could be important in the discovery of new drugs for the treatment of various neurodegenerative disorders (for review, 39, 228).

3. Cell models in the study of neuronal apoptosis and oxidative susceptibility. At present, there are no ideal experimental models of neurodegeneration that totally represent the human diseases. This notwithstanding, a variety of animal and cell models have been developed by different investigators to examine specific gene-based and cellular/metabolic-based factors that contribute to the neurodegenerative process. In this section, we will discuss the usefulness of cell culture models in the study of neuronal apoptosis, a recognized central component in neurodegeneration. The discussion of disease-specific animal models that have been developed to closely resemble specific neuropathology will be considered in the respective sections on the different neurodegenerative diseases (Sections III.B.1 to III.B.6).

Neuronal cell lines. As an experimental tool, neuronal cell cultures offer several advantages that include ease of use, relatively low expense, rapid data generation, and importantly, a simple system that allows for extensive and broad manipulation of experimental conditions that is not feasible in animal models. Unfortunately, *in vitro* cell models for the specific study of neurodegeneration are not available because brain neurons and motor neurons in patients with neurodegenerative disorders cannot be cultured successfully. To investigate the cellular and molecular mechanism(s) that are relevant to the neurodegenerative process, investigators have developed a number of alternative neuronal cell models of human and animal origin. The most popular cell models of human origin include the neuroblastoma cell lines, SH-SY5Y and SK-N-SH, the teratocarcinoma cell line, Ntera 2 (NT2), and the embryonic kidney cell line, HEK293. Neuroblastoma cells resemble sympathetic neuroblasts while NT2 cells resemble the committed CNS neuronal precursor cells (293). The HEK293 cell line, despite its kidney origin, expresses neuronal markers and likely possesses neuronal lineages (331). Among the more commonly used neuronal cell lines of non-human origin include the rat adrenal pheochromocytoma (PC12) cell line, the mouse neuroblastoma cell line Neuro-2a (N2a), and the murine hippocampal cell line, HT22. Primary rat cortical and hippocampal neurons isolated from rat embryos are also models of choice among researchers. For studies that mimic neurodegeneration affecting motor neurons, a hybrid cell line, NSC-34, has been generated; this cell line was produced by the fusion of motor neuron-enriched embryonic mouse spinal cord cells with mouse neuroblastoma cells (46).

The PC12 cell line is a popular choice in the study of neuronal apoptosis in different laboratories given the ease with which they can be grown in culture and the fact that they readily differentiate into neuronal-like cells. A notable feature of PC12 cells is that, when differentiated, neuron-like PC12 cells can produce and release catecholamines, such as dopamine and noradrenaline, which closely resemble the dopaminergic neurons of the sympathetic nervous system. Indeed, the fact that PC12 cells can easily transition from naïve to differentiated states with addition of NGF presents us with a suitable model to study the vulnerability of naïve and differentiated phenotypes to oxidative challenge. Our results show that by inducing PC12 differentiation, we obtained a phenotype that was significantly more resistant to *tert*-butyl-hydroperoxide (*t*BH)-induced oxidative damage, and that this resistance corresponded to a highly reduced intracellular GSH redox environment, decreased expression of Apaf-1, and preservation of the $\Delta\psi$m (87, Fig. 6). We subsequently established that the decreased susceptibility of differentiated PC12 cells is a generalized phenomenon in response to other oxidative challenges such as carbonyl stress (262) and hyperglycemic stress (263, 264). In other studies, Sung *et al*. (355) similarly found that neuron-like differentiated PC12 cells were less sensitive to H_2O_2 than naïve PC12 cells, while Wright *et al*. (399) showed that the differentiation of PC12 cells was accompanied by a marked reduction in Apaf-1 expression and a significant decrease in apoptosome activity. Decreased vulnerability of the differentiated phenotype to toxin exposure was also observed for Paju cells, an NGF-independent human neuroblastoma cell line, as evidenced by the cells' ability to maintain their $\Delta\Psi$m and to increase the expression of Bcl-2 and the neuroprotective factor, stanniocalcin (366). Interestingly, the acquisition of oxidative resistance associated with neuronal maturation and differentiation appears to be a physiological process as evidenced by the downregulation of Bax during postnatal brain development and an age-related decrease in the sensitivity of brain mitochondria to BH3-peptide-induced cytochrome *c* release (295). The mechanism governing neuronal cell transition (*e.g.*, differentiation) is unclear; in

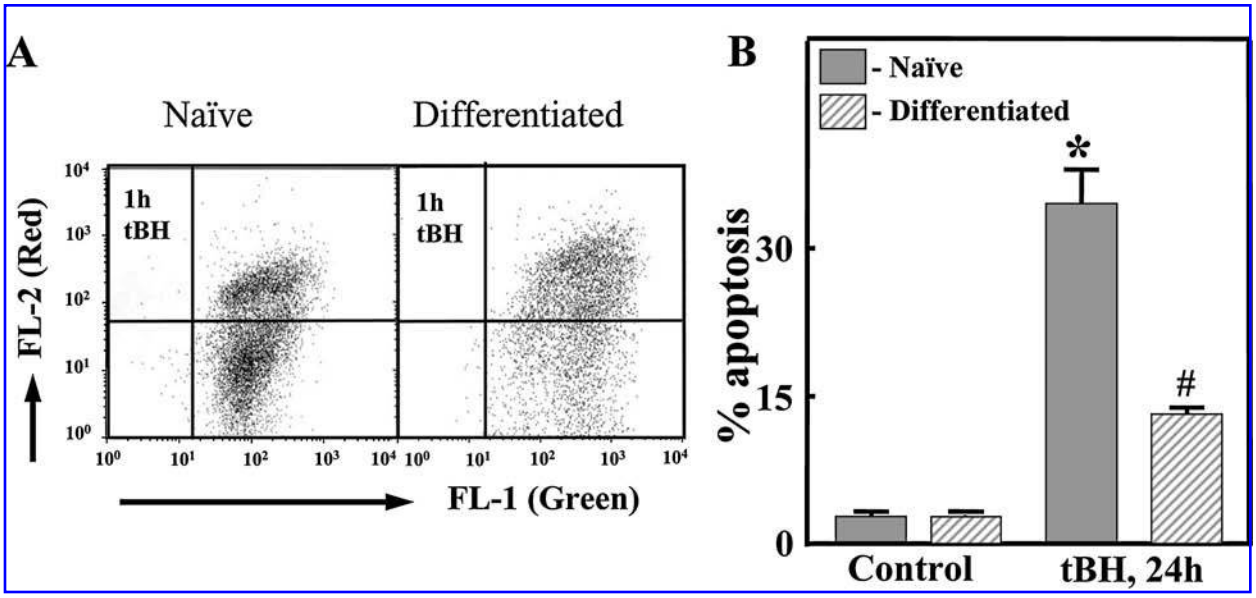

FIG. 6. Differential vulnerability of naïve and differentiated PC12 cells to oxidative challenge. Naïve PC12 cells are more sensitive to apoptosis induced by *tert*-butylhydroperoxide (*t*BH) as evidenced by a loss of $\Delta\psi$m measured by JC-1 fluorescence and flow cytometry (**A**) and increased number of cells exhibiting nuclear morphology characteristic of apoptosis measured by DAPI staining (**B**). The results are redrawn from (87).

intestinal cell lines, chemical-induced differentiation was accompanied by oxidation of the cellular GSH/GSSG redox state (255), suggesting the involvement of ROS and redox.

While many studies support the view that the differentiated neuronal phenotype is associated with oxidative resistance, an increased sensitivity of differentiated neuronal cells to oxidative challenge has been reported. For instance, Sasaki *et al*. (322) demonstrated that differentiated PC12 cells were more sensitive to various hydroperoxides (H_2O_2, *t*BH, linoleic acid hydroperoxide), in association with decreased GSH peroxidase activity and cellular GSH. Similarly, Wang *et al* (392) showed that neuronally differentiated human NT2/D1 cells exhibited greater sensitivity to H_2O_2 that was correlated with an eightfold decrease in intracellular GSH. Differences in culture conditions, including serum content and duration of exposure to differentiating agent (*e.g.*, NGF) are likely contributing factors to the disparate results of oxidative susceptibility between differentiated and naïve phenotypes observed in different laboratories. Nonetheless, the suggestion that the differentiated neuronal phenotype can exhibit higher resistance to oxidative stress may have important implications for understanding the etiology of the neurodegenerative process. Neuronal dendritic atrophy and disturbed NGF-induced neurite extensions, characteristics of a de-differentiated phenotype, have been observed in the neuropathology of AD and diabetic encephalopathy (9, 222). If the pathological loss of the differentiated phenotype is, indeed, a feature of neurodegeneration, the neurons would then be vulnerable to oxidative challenge; however, whether an elevated oxidative vulnerability of the undifferentiated (naïve) state of neuronal cells directly contributes to enhanced neuronal death (apoptosis) and disease pathogenesis remains to be determined (262). Although terminally differentiated mature neurons appear to exhibit a prosurvival phenotype, they do retain the normal full apoptotic machinery and ability to undergo apoptosis in response to various apoptotic stimuli.

B. Neurodegenerative diseases

The quantitative importance of neuronal apoptosis to the neurodegenerative process remains unresolved since much of the evidence implicating a role for apoptosis was derived from data using the classical criteria for the definition of apoptosis, based on morphological assessment and biochemical assays that are often not unique to apoptosis. Moreover, data interpretation is further complicated by contributions of other forms of cell death, such as "programmed necrosis" that appear to share similar death characteristics and/or features with apoptosis (see Section II.A). Despite these limitations, there is consensus for apoptotic participation in neuronal cell death and the neurodegenerative process. It is noteworthy that many investigators have reported the existence of oxidative stress alongside that of neuronal apoptosis. Even though increased ROS production can induce apoptosis (see Section II.C.1), there is no compelling evidence at present that causally link the two events in the neurodegenerative process. In the following sections, we have summarized some of the evidence in the better-studied neurodegenerative diseases of AD, PD, ALS, SMA, HD, and diabetic encephalopathy where there exists clear evidence suggestive of apoptosis involvement in neuron loss. Without implicating a causal link between oxidants and neuronal apoptosis, we have also included discussion of oxidative stress, where relevant, given the copious amount of evidence for the association of oxidative damage and neurodegenerative diseases in the literature.

Neurodegeneration is associated with selective neuron loss. The two regions of the brain that are especially vulnerable to oxidative damage are the hippocampus and the substantia nigra (SN); hippocampal degeneration is characteristic of diabetic encephalopathy and AD, while SN degeneration is characteristic of PD (170). Selective neurodegeneration in AD and PD is linked to selective increases in indices of ROS-induced damage in these brain regions, respectively (3, 118). Consistent with their oxidative susceptibility, the antioxidant defense systems, which include enzymes such as SOD, GSH peroxidase (GPX), catalase, as well as GSH and vitamin E, are notably low in the hippocampus and SN as compared to other brain regions (170). Furthermore, in PD, most of the motor symptoms are due to selective depletion of dopaminergic, neuromelanin-containing neurons of SN. The occurrence of neuromelanin and its interaction with elevated iron, renders dopaminergic nigral neurons peculiarly susceptible to oxidative stress (92). The melaninization of the SN dopaminergic neurons appears to be critical in PD degeneration (85). Motor neurons, which are selectively affected in ALS, are highly specialized, large cells. Their size requires significant metabolic input for the maintenance of cellular transport and the membrane potential along the length of the axon. The side effect of this enhanced mitochondrial activity is increased ROS production. Potential intensification of ROS generation by mutant SOD1 makes the ALS motor neurons highly susceptible to ROS-induced damage (20).

1. Alzheimer's disease. Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with progressive deterioration of memory and intellectual function, resulting in dementia. Distinctive features of AD are extracellular senile plaques composed of aggregated amyloid β-peptide deposits and intracellular neurofibrillary tangles composed of neurofilaments and hyperphosphorylated tau protein. These histopathological hallmarks of the disease are observed in the neocortex, hippocampus, and other subcortical regions of AD patient brains essential for cognitive function. Most cases of AD are identified as "sporadic" with no clear family history. Familial ADs are rare, have an autosomal dominant nature, and are caused by mutations of the amyloid precursor protein (APP), presenilin 1, or presenilin 2 genes (330).

a. Occurrence of apoptosis and oxidative stress in AD brain. The involvement of apoptosis in AD brains was suggested by findings of DNA fragmentation as detected by TUNEL staining with concomitant upregulation of pro-apoptotic Bax protein (353), c-Jun protein (6), and effector caspase 3 (312). Whereas positive TUNEL staining is often equated with cell apoptosis, the results are inconclusive, given that the TUNEL assay also detects DNA breaks that are often characteristic of cell necrosis. More compelling evidence of apoptotic involvement comes from studies of Rohn and colleagues (312) who demonstrated the activation of mitochondrial and receptor-mediated apoptotic pathways in AD hippocampal brain sections wherein active caspase 9 was co-localized with active caspase 8. Moreover, the distribution of caspase-cleaved fragments of tau suggests that the activation of caspases preceded the formation of neurofib-

rillary tangles in brains of AD patients. In addition, the intracellular amyloid β peptide1-42 (Aβ(1-42)) has been shown to induce human neuronal cell apoptosis through Bax activation that resulted in cytochrome *c* release and activation of caspase 6 (417). While the relationship between oxidative stress and apoptosis in AD remains unresolved, evidence of oxidative stress has been reported alongside neuronal apoptosis. For instance, enhanced formation of lipid peroxidation products, like 4-hydroxynonenal (HNE), were found in the brain and cerebrospinal fluid of AD patients (217, 232), and elevated oxidized DNA bases in certain regions of AD brains (221). This increased oxidative stress was associated with decreased glutathione *S*-transferase (GST) activity (217). Interestingly, expressions of other antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase were elevated in hippocampus and inferior parietal lobule, indicating a possible protective gene response to increased peroxidation in the brain regions affected by the disease (4). Suggestion of a linkage between oxidative stress and disease genesis comes from a randomized, blinded clinical trial wherein oral intake of vitamin E slowed the disease progression (319).

b. Amyloid precursor protein, presenilins, and β-amyloid production. Most studies link the pathogenesis of AD with increased production and/or deposition of β-amyloid peptide in the brain. β-Amyloid is derived from the amyloid precursor protein (APP) through sequential proteolytic cleavage by β-secretase at the N-terminus of the Aβ domain of APP. The generated fragments are further processed by γ-secretase to yield β-amyloid. Alternatively, APP is cleaved by α-secretase, yielding a large soluble amyloid protein. Since α-secretase cuts APP within the Aβ sequence, its activity prevents the generation of β-amyloid peptide. Presenilins are involved in γ-secretase-mediated proteolytic cleavage of the C-terminal transmembrane fragments of APP after their generation by β-secretase (330). Mutations of APP and presenilins genes associated with familial AD tip the balance of APP processing in favor of neurotoxic β-amyloid. The pathogenesis of AD can thus be viewed as a shortage of neuroprotective soluble form of amyloid and a surplus of neurotoxic β-amyloid (Fig. 7). APP, a ubiquitously expressed protein, is abundant in neurons. The soluble form of APP is neurotrophic, neuroprotective, and anti-apoptotic (37, 160, 369, 377); it protects cells against apoptosis induced by endoplasmic reticulum stress (175), staurosporine, UV, and p53 (404). Although the biological function of APP is not fully understood, several studies strongly implicate its role in transcriptional regulation (42, 174). Overexpression of wild-type APP in PC12 cells induces the overexpression of MnSOD, cytochrome c, and catalase, and downregulates the basal and UV-induced c-Jun/JNK activity. The resultant decrease in JNK signaling prevented caspase 3 activation and attenuated UV-induced apoptosis. Furthermore, wild-type APP activates the prosurvival PI3K/Akt kinase and p42/p44 ERK1/2 MAP kinase pathways, which mediate the neuron survival-promoting action of BDNF (54). The overexpression of APP carrying the Swedish mutation failed to protect against UV-induced apoptosis (174), consistent with a role for APP in cytoprotection.

c. β-amyloid-induced oxidative stress and JNK activation. That β-amyloid production is associated with the generation of neuronal oxidative stress is well documented. For instance, β-amyloid induced the formation of hydroxyl and lipid radicals in PC12 cells, presumably due to the interaction of Aβ with transition metals (131). The specific Aβ(25–35) and Aβ(1–40) peptides have been shown to induce ROS production, lipid peroxidation, and decreased cell GSH that collectively impair mitochondrial respiratory function and deplete intracellular ATP (281). In human teratocarcinoma cells, treatment with Aβ(25–35) leads to elevated ROS generation, protein and lipid oxidation, decreased cell GSH and GSH reductase activity, and increased caspase activation. Notably, the exposure of human teratocarcinoma ρ^0 cells that lack functional mitochondria to Aβ(25–35) did not elicit oxidative changes, suggesting that the mitochondrion is the principal ROS source in β-amyloid-induced neurotoxicity (36). Not surprisingly, β-amyloid induces mitochondrial oxidative DNA damage (33). Whether these are potential mechanisms by which β-amyloid induces neuronal apoptosis are unclear. β-Amyloid-induced apoptosis has been demonstrated in various neuronal cell lines and primary cultures of cortical neurons, and involves the activation of the JNK and p38 kinase pathways. Because β-amyloid induces an early generation of H_2O_2 and HNE, and these ROS species fully

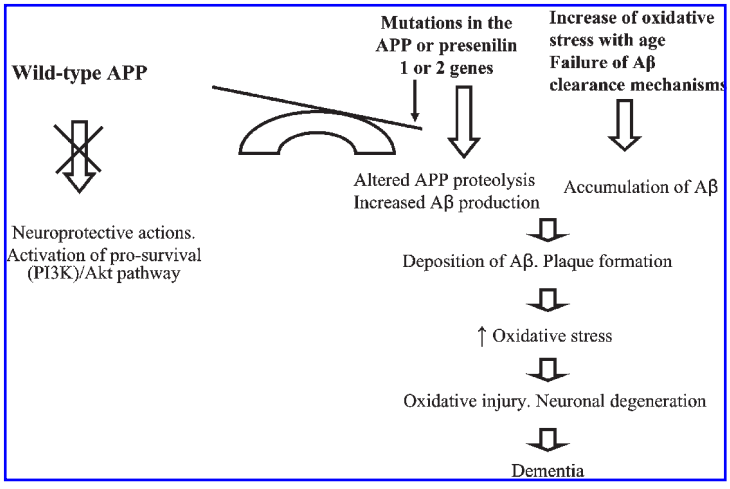

FIG. 7. A working model of Alzheimer's disease pathogenesis. The model proposes that AD-associated mutations of the APP and presenilin genes, as well as age-related increase in oxidative stress, tip the balance of APP processing toward formation of neurotoxic β-amyloid and/or accumulation of Aβ. A shortage of neuroprotective soluble amyloid, and a surplus of neurotoxic β-amyloid lead to neuronal degeneration and AD manifestation.

mimic Aβ-dependent activation of JNK and p38 and elicit characteristic apoptotic nuclear changes, Tamagno *et al.* have suggested a link between ROS and β-amyloid-induced neuronal apoptosis (359). In other studies, the overexpression of the Swedish double mutation in PC12 cells facilitated JNK activation after H_2O_2 treatment and rendered cells more vulnerable to oxidative stress-induced apoptosis (234). Mechanistically, JNK was shown to downregulate anti-apoptotic Bcl-xL and Bcl-w expression, and suppression of Bcl-w potentiated the release of Smac into the cytosol where Smac promoted caspase 9 activation. Accordingly, Bcl-w overexpression and JNK inhibition effectively prevented these β-amyloid-induced changes (409). Taken together, these findings suggest that oxidative stress and JNK activation are associated with β-amyloid-induced apoptosis. Given the many metabolic pathways that are mediated by ROS and MAP kinase signaling, it should be emphasized that apoptosis is only one of many biological endpoints.

Antioxidants can effectively protect against the oxidizing effects of β-amyloid (33, 43, 281). Studies by Akterin and colleagues (5) suggest that endogenous GRX 1 and TRX 1 afford the first line of defense against β-amyloid-induced oxidative stress and the apoptotic cascade (Fig. 8). Interestingly, while TRX1 in AD brains was downregulated, GRX1 was upregulated. These authors suggested that the upregulation of GRX1 could be a compensatory response to counteract the reduction in TRX1, since neurons with high expression of GRX1 in AD brains exhibited apparent normal morphology. Human SH-SY5Y neuroblastoma cells treated with Aβ(1–42) peptide elicited a strong, early, and transient oxidation of GRX1 and TRX1, which preceded GSH depletion. Apart from their role as antioxidants, GRX1 and TRX1 are capable of regulating apoptosis. Reduced GRX1 and TRX1 bind apoptosis signal-regulating kinase 1 (ASK1), and prevent the activation of the pro-apoptotic JNK and p38 signaling pathways. Oxidation of GRX1 and TRX1 leads to their dissociation from ASK1 and allows the initiation of the apoptotic cascade (346). Indeed, it was shown that β-amyloid activated ASK1 and JNK as early as 1 h after treatment, which corresponded to the kinetics of GRX1 and TRX1 oxidation. This activation is ROS-dependent since antioxidants such as ROS scavengers (propyl gallate), SOD mimetics, and $ONOO^-$ scavenger (MnTBAP), and vitamin E were effective in inhibiting β-amyloid-induced ASK1 activation. Moreover, β-amyloid failed to activate JNK in primary neuronal cell cultures derived from ASK1 knockout mice, and β-amyloid-induced cell death was significantly attenuated in $ASK1^{-/-}$ primary neuronal cultures. However, the inhibition of β-amyloid-induced neuronal death in $ASK1^{-/-}$ was incomplete, and therefore implicated the existence of additional ASK1-independent cell death pathways (153).

d. Effect of JNK and ERK on β-amyloid production. While current evidence supports β-amyloid-mediated activation of JNK, other evidence shows that β-amyloid production itself is regulated by JNK. Pro-inflammatory cytokines (IFN-γ, IL-1β, and TNF-α) can stimulate γ-secretase cleavage of APP through a signaling cascade mediated by MEKK1 and JNK. This finding provides the functional link between microglia-mediated inflammation, which enhances γ-secretase-dependent β-amyloid production, and β-amyloid-elicited neurotoxicity in the AD brain (207). γ-Secretase activity is negatively regulated by ERK, possibly by phosphorylation of nicastrin, a component of the γ-secretase complex. Thus, ERK1/2 activation appears to serve a protective role in AD by attenuating γ-secretase-dependent β-amyloid production (169).

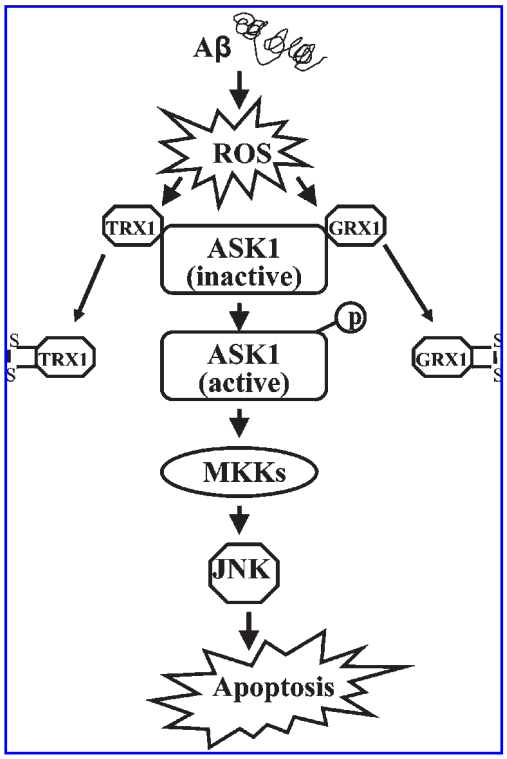

FIG. 8. β-amyloid-induced ASK1/JNK activation and apoptotic signaling. Neuronal β-amyloid-induced apoptosis follows a generalized scheme of ROS-induced apoptosis. β-Amyloid-generated ROS cause oxidation of TRX1 and GRX1, which dissociate from ASK1 and trigger ASK1/JNK pro-apoptotic pathway (see Fig. 3 for a detailed scheme of ASK1/JNK signaling involving participation of the ASK1-signalsome complex and various pro-apoptotic protein).

e. Neurofibrillary tangles. Activation of MAP kinases (ERK, JNK and p38) has been demonstrated in AD brain tissues. Differential staining for these kinases revealed that activated JNK and p38 are exclusively associated with neurofibrillary tangles, senile plaque neurites, granulovacuolar degeneration, and neuropil threads in severe AD cases. These findings suggest a link between β-amyloid-induced MAP kinase activation, JNK/p38-dependent phosphorylation of tau, and the formation of neurofibrillary tangles (420). This suggestion is supported by studies of brain sections in APP mice wherein activated JNK and p38 kinases co-localized with hyperphosphorylated tau in neuritis surrounded by β-amyloid deposits (298). Since ERK was not associated with the most severe pathological features in the AD brain (420), its activation may in fact be compensatory and affords resistance of certain neurons to β-amyloid-mediated cytotoxicity. Co-incubation of neurons with Aβ(1–42) peptide induced proteolysis of tau, indicating that tau can be modified by caspase cleavage. Tau cleavage was caspase-dependent since

TABLE 2. COMPARISON OF TRANSGENIC MURINE MODELS IN ALZHEIMER'S DISEASE PATHOLOGY

Name	*Genes(s) overexpressed*	*Neuropathology plaques*	*P-tau*	*NFT*	*Cell loss*	*Memory deficits*	*Age of onset (of pathology)*
A. Amyloid pathology mouse models							
PDAPP mice	App minigene. V717F mutation	Yes	Yes	No	No	Yes	6–8 months
Tg2576 mice	APP Swe cDNA(695)	Yes	Yes	No	No	Yes	9–11 months
APP23 mice	APP Swe cDNA (751)	Yes	Yes	No	Yes	Yes	6 months
TgCRND8 mice	APP cDNA Swe and V717F mutant	Yes	nr	No	nr	Yes	3 months
APPSwe TgC3-3 mice	App cDNA (695) Swe	Yes	nr	nr	nr	nr	18 months
PSAPP mice	Tg2576 and PS1 M146L	Yes	Yes	nr	Minor	Yes	6 months
Tg478/1116/11587 rat	APP Swe, APP Swe and V717F, PS1 M146V	Yes	nr	nr	nr	nr	9 months
B. Tau transgenic mouse models							
ALZ mice	4R tau	No	Yes	No	No	nr	–
ALZ17 mice	4R tau	No	Yes	No	No	nr	–
7TauTg mice	3R tau	No	Yes	Yes	nr	nr	18–20 months
JNPL3 mice	4R tau and P301L	No	Yes	Yes	Yes	Yes	5 months
pR5 mice	4R tau and P301L	No	Yes	Yes	Yes	nr	8 months
C. Combined models							
TAPP mice	Tg2576x JNPL3	Yes	Yes	Yes	nr	nr	6 months
3xTg-AD mice	App (Swe), PS1 (M146V), tau (P301L)	Yes	Yes	Yes	nr	nr	3 months

nr, not reported; Swe, Swedish mutation; P-tau, phosphorylated tau immunoreactivity.

Different murine models have been developed to mimic the pathology in AD. These models are based on the overexpression of APP, presenilins, or tau to induce production and accumulation of β-amyloid into plaques or hyperphosphorylated tau into neurofibrillary tangles. The first mouse model to reproduce amyloid pathology was derived from overexpression of the amyloid precursor protein. The tau transgenic mouse models have been developed to model the pathology of neurofibrillary tangles in AD. Recently, combined mouse models that combine amyloid and tau pathology have been generated. (Revised from Ref. 349).

the broad spectrum caspase inhibitor zVAD-fmk effectively prevented its proteolysis. Moreover, apoptotic changes in the nuclei of Aβ(1–42)-treated neurons were associated with positive immunostaining for caspase-cleaved tau. Accordingly, neurons that were negative for caspase-cleaved tau rarely exhibited apoptotic nuclei. Cleaved tau in neurofibrillary tangles, and dystrophic neurites forming neuritic plaques have been detected by immunostaining in sections of human hippocampus from AD brain (99).

f. Animal models of Alzheimer's disease. Currently, no animal models fully represent the pathophysiological changes and symptoms of human AD. Nevertheless, studies of similar and related pathological processes in transgenic animals have provided important insights into disease mechanisms. Several of these models are based on the overexpression of APP, presenilins, or tau to induce production and accumulation of β-amyloid into plaques, or hyperphosphorylated tau into neurofibrillary tangles, both of which are key features of human AD (349) (Table 2). A comprehensive review of all existing models is beyond the scope of the current review; in the following sections we have limited our discussion to the more common murine models used in AD research.

APP transgenic mouse strains. The first transgenic mouse model of AD was generated to overexpress APP in an attempt to reproduce amyloid pathology. Although these mice showed considerable neuronal apoptosis, they did not exhibit the progressive increases in extracellular β-amyloid peptide or neurofibrillary tangle formation characteristic of human AD. To achieve these features, a familial AD (FAD) mutation was introduced in APP in mice overexpressing the protein, and models of transgenic mice, which express human familial Swedish double mutation, were developed, *viz.*, the Tg2576 and APP23 mice. However, not all transgenic mouse strains displayed characteristic neurodegenerative features of human AD even when high levels of β-amyloid were found in the central cerebral fluid. This could be attributed to a number of factors, most notably, the fact that mutant mice rarely exceed 24 months of age, while the neurodegenerative process in the brains of patients with AD develops over decades. It is entirely feasible that neurons can tolerate the effects of amyloid fragments for several years before neurodegeneration sets in. In addition, mutant mice do not express tau tangles, which in the human AD brain are toxic and can accelerate neuronal degeneration. This notwithstanding, the mutant APP mice carrying the Swedish mutation have been favored by investigators in AD research and have provided novel insights into the mechanism of disease pathogenesis.

Multiple transgenic mouse strains. To hasten the development of plaques and the physiological effects of mutated APP on neuronal metabolism and survival, second generation models of multiple transgenic mice have been developed which ex-

press the human familial double mutation of APP in combination with human mutated presenilin-1. Comparison of the Tg2576 mouse model containing the Swedish double APP mutation with the triple mutant PS1/APP^{Swe} transgenic mouse strain revealed that plaques developed by as early as 3 months in the triple mutant mice; in contrast, Tg2576 mice developed plaques by 6–9 months. At 4–5 months of age, PS1/APP^{Swe} mice exhibited significant impairment in water maze probe tests, while Tg2576 mice did not exhibit similar impairment until 6 months of age. This observation was validated in a study of such a triple mutation transgenic mouse strain carrying the Swedish (K670N/M671L) APP mutation with a FAD4 (M146L) mutant presenilin-1 line. Development of fibrillary β-amyloid deposits in these mice was observed by 6 months of age, with deficits in Y-maze alteration tasks by as early as 3 months of age (136).

Tau transgenic mice and combined models. To model the pathology of neurofibrillary tangles in AD, transgenic mice overexpressing wild-type tau (ALZ7, ALZ17 models) have been generated. Since overexpression of wild-type human tau *per se* is insufficient to induce neurofibrillary tangles formation and only replicates very few aspects of tau pathology of AD in mouse models, many investigators in AD research have turned to the newly discovered pathogenic tau mutations for use in animal model development (JNPL3 and pR5 mice). Oddo and coworkers have developed another transgenic mouse model that combines amyloid and tau pathology (259). Their 3xTg-AD mice harbor mutations of APP (Swedish), PS1 (M146V), and tau (P301L). These mice develop plaques first in the neocortex at around 3 months of age that spread to the hippocampus by 6 months. Moreover, the 3xTg AD mice develop cognitive problems by 6 months of age (28). The tangles develop amyloid pathology and appear first in the hippocampus at 12 months, and then spread to the cortex. These specific regional and temporal developments of pathology closely mimic the development of pathology in human AD. Thus, the tau transgenic mice combined with APP overexpression provide an excellent experimental tool for studying the mechanism of neuronal apoptosis in AD pathogenesis. Additionally, the availability of knockout models with AD associated proteins, *viz.,* APP, PS2, beta-site APP cleaving enzyme, insulin-receptor substrate-2, Aβ-degrading enzyme neprilysin, and ApoE, will be beneficial in exploring the native functions of these genes and their mechanistic role in AD development in the future.

2. Parkinson's disease. Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after AD. PD is characterized by a progressive degeneration of dopaminergic neurons in the SN pars compacta, resulting in typical signs of slowness of voluntary movements, resting tremors, rigidity, and gait imbalance. The pathological hallmark of PD is Lewy bodies, intraneuronal proteinaceous cytoplasmic inclusions composed of misfolded and aggregated α-synuclein protein (348). PD cases are generally of sporadic origin, and only ~5% are familial cases, which are usually early onset and more aggressive. Among the causative genes of the disease are α-synuclein, and leucine-rich repeat kinase 2 (LRRK2, alternative name: dardarin) that are responsible for autosomal dominant PD, and parkin, DJ-1, and PINK-1, that are responsible for autosomal recessive PD (102). Chemically induced rodent models of PD have been generated. Targeted injections of 6-hydroxydopamine (6-OHDA) are given to localize the damage to the SN site, producing lesions of the dopaminergic system. Unilateral intracerebral injection of the toxin is favored since bilateral injection often leads to animal mortality (31). 6-OHDA is primarily utilized to generate the rat model of PD, although mice and monkeys have also been used. Disadvantages of this chemical model of PD induction are the absence of PD-associated Lewy bodies in SN regions and nonspecific damage to nondopaminergic neurons (22). Currently, the most widely used mouse model of PD is one induced by the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 187). Rat dopaminergic neurons are relatively resistant to MPTP (31).

a. MPTP-induced parkinsonian syndrome. MPTP, a byproduct of the illicit manufacture of a synthetic meperidine derivative, induces a parkinsonian syndrome that is essentially indistinguishable from PD. MPTP is converted to MPP^+ ion by monoamine oxidase, is selectively taken up by the dopaminergic neurons via dopamine transporter, and selectively inhibits complex I of the mitochondrial respiratory chain (187). Subchronic exposure of mice to MPTP caused death of nigral dopaminergic neurons suggestive of apoptosis as evidenced by the appearance of double-stranded DNA breaks, morphological nuclei changes (363), and caspase 3 activation (376). The suggestion that MPTP induced an apoptotic process is supported by the findings that Bcl-2 overexpression (260) and Bax deficiency (388) prevented dopaminergic neurodegeneration. MPTP neurotoxicity was also attenuated by the administration of JNK-specific inhibitors (320), by overexpression of MnSOD (173), and by knockdown of nuclin, a novel protein shown to be an essential factor for apoptosome induction (365). Furthermore, transgenic mice with a knockdown of the pro-apoptotic p53 gene acquired resistance to MPTP neurotoxicity (370). Studies of MPTP-induced apoptosis in dopaminergic PC12 cells, SH-SY5Y neuroblastoma cells, and primary mesencephalic cells provided evidence of MPTP-induced ROS production, activation of p53, cleavage of caspase 3 and PARP (90, 129, 130), and JNK activation (400).

b. Oxidative stress in Parkinson's disease: dopamine and α-synuclein. A state of oxidative stress has been unambiguously identified in the SN of the PD brain as evidenced by increased lipid, protein, and DNA oxidation, as well as increased total iron contents (78, 79, 95). Of note are the significant decreases in GSH and the GSH/GSSG ratio in the SN region (336, 343). It is interesting that the dopaminergic neurons of the SN region of the normal brain exhibit high levels of basal oxidative stress, rendering this region vulnerable to toxic insults, such as that caused by the products of dopamine metabolism, acting as endogenous toxins. Dopamine is metabolized by monoamine oxidase which generates H_2O_2 (225), or is autooxidized to yield $O_2^{\cdot -}$ radicals, H_2O_2, and dopamine-quinone species (116). Thus, the redistribution of vesicular dopamine to the cytoplasm can be toxic to dopaminergic neurons. Whether dopaminergic neuronal death occurs by an apoptotic process under these conditions is unknown. A role for p38 and JNK MAP kinase in apoptotic signaling has been demonstrated (96, 219). In addition to oxidative challenge, the loss of essential growth factors critical for survival and differentiation of dopaminergic neurons

can also contribute to the pathogenesis of PD; the finding that levels of NGF and BDGF are attenuated in the SN regions of parkinsonian patients supports this suggestion (222). Other supporting evidence comes from cell studies where PI3K/Akt-mediated enhanced NGF activity was shown to protect against MPTP-induced PC12 apoptosis (335).

The suggestion that dopamine sequestration into synaptic vesicles protects nigral cells from toxic dopamine metabolites has been documented. Interestingly, gain-of-function polymorphisms in the vesicular monoamine transporter gene, which increase sequestration of dopamine into vesicles, was shown to have a selectively protective effect only in females (109). The fact that depletion of α-synuclein leads to a substantial decrease in the number of synaptic vesicles in the reserve pool and that hippocampal terminals in brains of α-synuclein-knockout mice exhibit impaired response to prolonged stimulation, suggests a possible role for α-synuclein in vesicle recycling via inhibition of phospholipase D2 (216). Lotharius and Brundin hypothesized that a mutation in α-synuclein leads to the reduction in vesicle number and an accumulation of cytoplasmic dopamine in association with enhanced ROS generation and initiation of the apoptotic cascade (216). Results of studies with wild-type α-synuclein overexpression are controversial. Some investigators showed that its overexpression leads to impairment of mitochondrial function, increased oxidative stress, and neurodegeneration in SN (151, 419), while others found that overexpression of wild-type α-synuclein confers resistance to oxidative stressors and apoptotic stimuli (161, 230). A recent study demonstrated that α-synuclein exerts its anti-apoptotic function by interacting with pro-apoptotic Bad and PKCδ (165). Importantly, α-synuclein has a tendency to form oligomers and fibrils that is facilitated by oxidative stress and α-synuclein mutation (67).

The availability of transgenic mouse models overexpressing the wild type or mutant α-synuclein has underpinned the advances in PD research. Unfortunately, these mice do not exhibit loss of dopaminergic nigrostriatal neurons and only minimally exhibit some signs of dopaminergic dysfunction and PD-like behavioral impairment. As such, these α-synuclein transgenic models are useful for studies on the early stages of PD (238).

c. Parkinson's-associated gene mutations: is mitochondrial disruption a common pathway in the pathogenesis of different types of familial PD? Mutations in the parkin gene are the most frequent cause of familial PD. Parkin is associated with the outer mitochondrial membrane (69) and is involved in protein degradation as an ubiquitin-protein ligase. Overexpression of wild-type parkin in neuronal cells was shown to be protective against serum withdrawal, H_2O_2, MPTP, or HNE-induced cell death, while overexpression of mutated parkin exacerbated cell apoptosis (141). Increased expression of wild-type parkin also protected neuroblastoma cells against dopamine-induced apoptosis by attenuating dopamine-induced activation of JNK and caspase 3 (147). The neurons of parkin knockout mice exhibit abnormalities in protein processing, impairment of dopamine metabolism, and increased ROS production (143). Although the relationship between these events has yet to be defined, the parkin knockout mice have been shown to exhibit greater susceptibility to rotenone toxicity than wild type mice (45). Another PD-associated gene, DJ-1, a mitochondrial oxidative stress-response chaperone, shows ubiquitous expression with higher levels of the transcript in the subcortical regions, including the SN site (30). Wild-type DJ-1 protects against oxidative-stress-induced cell death in part by quenching ROS, and in part, by sequestering the death protein DAXX in the nucleus, which prevents it from binding to cytoplasmic ASK1 and blocks the apoptotic cascade (152). Interestingly, knockdown of the DJ-1 gene elicits mild changes in dopamine metabolism and decreased locomotor activity in mice without significant loss of nigrostriatal dopaminergic neurons, while the DJ-1 mutation results in enhanced sensitivity to MPTP (168). All of the autosomal recessive PD-associated genes, parkin, DJ-1, and PINK-1 are involved in mitochondrial function, as are possibly the autosomal dominant PD-associated genes (α-synuclein and LRRK2). These findings, and the fact that PD toxins act by impairing mitochondrial activity, suggest that mitochondrial dysfunction, including the generation of ROS, is a central event in the pathogenesis of PD (360). Despite ample independently derived evidence of the involvement of oxidative stress and the existence of various apoptotic markers in PD, a causal relationship between oxidative stress and neuronal apoptosis in PD pathology remains an open question.

3. Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is a fatal disorder that is characterized by selective and progressive degeneration of upper and lower motor neurons. Approximately 10% of cases of ALS are familial, usually with autosomal dominant inheritance. Among them, ~20% involve mutations of the cytosolic Cu/Zn SOD (SOD1) gene (332).

a. Occurrence of oxidative stress, mitochondrial dysfunction, and apoptosis in ALS. Increased oxidative stress and mitochondrial dysfunction are well documented in ALS, as evidenced by elevated protein, lipid, and DNA oxidation both in transgenic mouse models of ALS, and in tissues of ALS patients (23, 32, 125, 254, 342). In addition, increased incidence of mitochondrial DNA mutation and decreased activities of mitochondrial respiratory chain complexes have been demonstrated in spinal cords of patients with sporadic ALS (397). Evidence of apoptosis such as DNA fragmentation, increased caspase 3 activity, and changes in the subcellular distribution of the pro-apoptotic Bax and Bak, and anti-apoptotic Bcl-2 proteins was also found in ALS spinal cord anterior horn and motor cortex (236). Moreover, the prostate apoptosis response-4 (Par-4) protein was elevated in the lumbar spinal cord samples from patients with ALS and in transgenic mice overexpressing mutant SOD1 gene with a G93A mutation. Whether these independent observations are mechanistically linked is unknown. In cell cultures, Par-4 was upregulated after exposure of cells to oxidative insults, and Par-4 antisense oligonucleotide treatment attenuated mitochondrial dysfunction and oxidative stress-induced apoptosis (278).

Involvement of apoptosis in ALS-associated neuronal death was supported by the findings of decreased Bcl-2, and increased Bax levels in the lumbar cords of ALS patients and transgenic SOD^{G93A} mice (251, 390). A delay in the degeneration and death of motor neurons in transgenic mice overexpressing the mutant SOD1 gene with a G93A mutation was associated with caspase inhibition and Bcl-2 overexpression (179, 197). Since

these mice exhibit clinicopathological features that are characteristic of familial ALS, *viz*., progressive hind limb weakness and neurogenic amyotrophy, appearance of SOD1-immunoreactive inclusions in the lower motor neurons, and a decreased number of motor neutrons in the advanced stage of the disease (334), they provide useful tools for the study of apoptosis in ALS genesis. In cell studies, overexpression of several mutant SOD1s (A4V, G37R, G93A, and V148G) promotes apoptosis in a variety of mammalian neural cells (299), including primary neurons from transgenic mice (242), differentiated PC12 cells, superior cervical ganglion neurons, and hippocampal pyramidal neurons (106). Copper (Cu^{2+}) chelators, Bcl-2, caspase inhibitors, and antioxidants such as GSH and vitamin E prevent mutant SOD1-induced apoptosis of hippocampal neurons, which is consistent with involvement of oxidative stress in neuronal apoptotic death. Participation of the intrinsic apoptotic pathway in mutant SOD1-induced neuronal death has been demonstrated; overexpression of mutant SOD1s (G93A, G37R, G85R, and I113T) in neuronal cell lines resulted in cytochrome *c* release and caspase 3 and 9 activation (68). Moreover, overexpression of Apaf1 exacerbated apoptosis in SOD1 mutant cells, while deletion of Apaf1 prevented SOD1-induced caspase activation and apoptosis (68).

b. SOD1-dependent downregulation of nuclear factor-erythroid 2 p45-related factor 2 and antioxidant response element-responsive genes, and Fas/NO-mediated motor neuron death. Expression of mutant G93A SOD1 results in transcriptional repression; of the 268 genes examined, expression of 197 genes were decreased. Key among the downregulated genes are the transcription factor, nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), and phase II detoxifying enzymes, the expression of which is regulated by Nrf2 through the antioxidant response element (ARE) (171). Activation of ASK1, p38, MKK3/6 (known activator of p38), nNOS, and caspase 3 was demonstrated in motor neurons of the spinal cord and cerebral cortex of mice overexpressing mutant G93A SOD1. It was shown that Nrf2 is an inhibitor of Fas-induced apoptosis (180, 249). Thus, the mutant SOD1-induced downregulation of Nrf2, which leads to the activation of Fas-mediated apoptosis, may represent a novel apoptotic pathway that is unique to motor neurons. Raoul *et al*. (301) demonstrated that, apart from inducing the classical FADD/caspase 8 cascade, this motor neuron-specific Fas-mediated death pathway requires transcriptional upregulation of nNOS that involves Daxx, ASK1, and p38 activation. Curiously, p38 MAP kinase activation was not accompanied by the activation of JNK (135, 394, Fig. 9), hence, the investigators have suggested that activation of p38 MAP kinase rather than JNK, is unique to death signaling in motor neurons in ALS (135, 301, 394). Importantly, this finding suggests that activation of different MAP kinases could contribute to the specificity of ROS-mediated death signaling among the different neurodegenerative diseases, such as AD and ALS.

Motor neurons from transgenic mice overexpressing mutant $SOD1^{G93A}$ or $SOD1^{G85R}$ exhibit increased sensitivity to the activation of this motor neuron-specific pathway. Mutant SOD1 cells are more sensitive than wild-type motor neurons to Fas- or NO-triggered cell death but not to trophic deprivation or excitotoxic stimulation. Uniquely, exogenous NO induces Fas ligand upregulation and Fas activation in motor neurons bearing mutant $SOD1^{G93A}$ and/or $SOD1^{G85R}$, but not the wild-type SOD1. A subsequent Fas-activated FADD/caspase 8 apoptotic cascade led to Daxx-mediated p38 phosphorylation and increased NO synthesis, thus creating a Fas/NO feedback amplification loop (Fig. 9) (300).

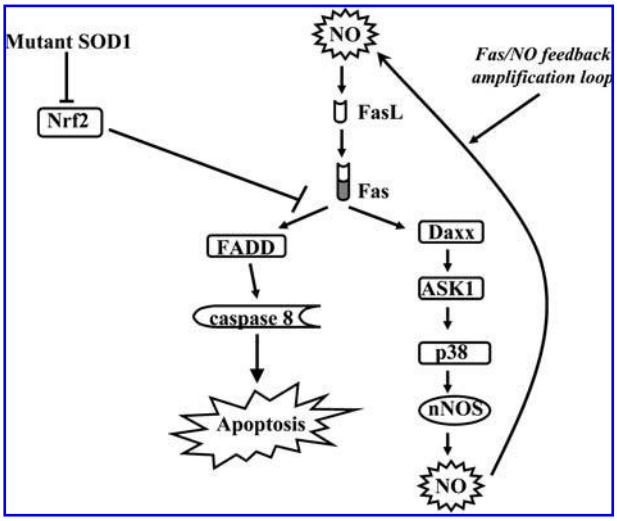

FIG. 9. Mechanism of mutant SOD1 neurotoxicity in ALS. Fas-mediated apoptosis of motor neurons involves induction of the classical FADD/caspase 8 cascade, as well as motor neuron-specific transcriptional upregulation of nNOS and activation of Daxx, ASK1, and p38 activation. Chronic low-level activation of Fas/NO feedback loop leads to the death of motor neurons. Notably, the activation of p38 MAP kinase is specific to motor neurons; JNK activation is not involved. Mutant SOD1 is purported to downregulate Nrf2 and ARE-driven genes, which removes the inhibitory effect of Nrf2 on Fas-mediated apoptosis.

c. Gain of toxic function of mutant SOD. The major function classically attributed to SOD1 is the conversion of $O_2^{\cdot -}$ to H_2O_2. However, considerable evidence shows that mutant SOD1 has a gain of toxic function, but the mechanism of this enhanced toxicity is elusive. Notably, most of the SOD1 mutations do not result in reduced SOD1 activity, as evidenced by normal enzyme activity in transgenic mice overexpressing SOD^{G37R}, $SOD1^{G85R}$, and SOD^{G93A} (36, 307). Moreover, SOD-deficient mice develop normally and exhibit no evidence of motor neuron cell death by 6 months of age (303), while mice carrying the familial ALS mutation $SOD1^{G93A}$ exhibit motor neuron degeneration beginning at 3–5 months of age (122). One hypothesis that explains the gain of toxic function is that mutant SODs can directly promote generation of reactive oxygen and nitrogen species. Another hypothesis proposes that mutant SODs are prone to aggregation due to instability or association with other proteins (210).

The clinical significance of a gain of toxic function to ALS development is unclear. This notwithstanding, it is tempting to speculate that the gain of toxic function of mutant SOD could underlie the mechanism of mutant SOD1-induced downregulation of Nrf2-dependent transcription. Certain familial ALS mutations (A4V, G37R, G41D, and G93A) increase the de-nitro-

sylase activity of SOD1 and disrupt the function of proteins that are regulated by *S*-nitrosylation (325). Although in some instances *S*-nitrosylation, such as the *S*-nitrosylation of GAPDH described in Section III.A.2, may lead to induction or exacerbation of apoptotic cell death, de-nitrosylation can be deleterious as well. For instance, *S*-nitrosylation protects Bcl-2 against proteosomal degradation; inhibition of Bcl-2 *S*-nitrosylation increased its ubiquitin-proteasomal degradation and promoted chromium-induced apoptotic cell death (12). Moreover, since *S*-nitrosylation is required for the anti-apoptotic function of TRX (124) and is known to inhibit caspase activation (158), de-nitrosylation of these proteins can promote apoptosis. Indeed, activation of the Fas apoptotic pathway was associated with denitrosylation of caspase 3 zymogens, which consequently resulted in an increase in intracellular caspase activity (229). Since motor neurons, carrying the mutant SOD1 gene are more sensitive to Fas-mediated apoptosis than are wild-type motor neurons (300), it is conceivable that mutant SOD1-mediated impairment of *S*-nitrosylation may affect normal cell signaling and the cell's ability to activate antioxidant defense mechanisms, and therefore promote apoptotic death of motor neurons.

4. Spinal muscular atrophy. Spinal muscular atrophy (SMA) is a neurodegenerative disorder characterized by the loss of the α-motor neurons of the spinal cord, resulting in progressive atrophy of the voluntary muscles of the limbs and trunk. SMA is the second most common autosomal recessive disorder, with a prevalence of 1 in 10,000 live births. SMA is caused by mutations of the survival motor neuron (SMN)1 gene. A second adjacent, highly homologous gene, SMN2 has a five nucleotide difference compared to SMN1. SMN2-derived transcripts are prone to alternative splicing, which leads to the excision of exons 5 and/or 7 and to generation of a truncated protein (191). The severity of the disease correlates with the deletion of a neighboring gene, the neuronal apoptosis inhibitory protein (NAIP) gene (313).

a. Enhancement of naturally occurring neuronal death in SMA and anti-apoptotic properties of NAIP and SMN. It is notable that among all neurodegenerative diseases, that the pathogenesis of SMA involves an exacerbation of naturally occurring death of motor neurons (321), as evidenced by an increase in the number of TUNEL-positive motor neurons in SMA fetuses at 12 and 15 weeks of gestational age. Moreover, all fetal and neonatal SMA samples showed a decreased number of motor neurons compared to age-matching controls, supporting the idea of an increase in naturally occurring neuronal death as a main cause of disease development (345). The fact that NAIP gene deletions is correlated with disease severity and that the protein family of inhibitors of apoptosis to the homology of NAIP suggests an apoptotic involvement in disease genesis. Numerous studies have revealed anti-apoptotic properties of NAIP in different neuronal cell lines and primary cultures, and in animal models of neurodegenerative disorders (115, 193, 212, 339). Maier and colleagues (223) demonstrated that NAIP acts as a direct inhibitor of effector caspases 3 and 7. Full-length wild-type SMN also exhibits anti-apoptotic properties; it protects neuronal PC12 cells against apoptotic death induced by trophic deprivation and UV treatment, and delays the onset of caspase activation and apoptosis after trophic factor withdrawal by inhibiting the release of cytochrome *c*. Furthermore, overexpression of SMN in PC12 cells significantly decreased the percentage of cells exhibiting nuclear p53 immunostaining after UV treatment as compared to control cells (391). Mechanistically, the anti-apoptotic properties of full-length SMN can be explained by its interaction with Bcl-2 and p53. Bcl-2 and SMN proteins bind to each other at the BH4 domain of Bcl-2 and the exon 6 region of the SMN gene (323) which synergistically attenuates Bax- and Fas-mediated apoptosis (144).

A pro-apoptotic effect of mutant SMN against Sindbis virus-induced apoptotic death has been documented *in vitro* in primary neurons and differentiated neuron-like stem cells, and *in vivo* in newborn mice (162). Importantly, the region encoded by exon 6 of the SMN gene is the most evolutionarily conserved, and the majority of missense mutations of the SMN gene in SMA patients occur in this exon. For instance, SMN^{Y272C}, which carries a missense mutation in exon 6, fails to elicit synergistic anti-apoptotic effect with Bcl-2 (144). The SMN/p53 association is mediated through the region encoded by SMN exon 2, and mutant SMN proteins display a dramatically reduced ability to bind p53. Significantly, the decrease in the ability of different mutant SMN proteins to bind p53 correlates with disease severity (413).

The evolutionary duplication of the SMN gene, SMN2, is unique to humans (311). Mice have one SMN gene, and its elimination leads to embryonic lethality (326). To mimic the human gene pattern in a mouse model, the human SMN2 gene was introduced into the Smn-deficient mouse genome, which rescued the $Smn^{-/-}$ mice from lethality. SMA mice thus generated possess a phenotype that is similar to that seen in SMA patients (247), and exhibit significant increases in pro-apoptotic Bax expression in the spinal cord. Transgenic SMA mice with an additional knockout of Bax gene showed milder disease severity and a significant increase in the density of motor neurons (372). It is anticipated that the availability of these murine models will spur future research in the mechanistic relationship between motor neuron apoptosis and SMA neuropathology *in vivo*.

b. Mutations of housekeeping SMN gene—a cause of motor neuron-specific disease. SMN is a housekeeping gene. The SMN protein is localized to the cytoplasm diffusely, and to the Cajal bodies in the nucleus where it plays an essential role in the biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs) and therefore in pre-mRNA splicing (280). Interestingly, p53-inducing stimuli causes the accumulation of p53 in SMN-positive bodies, while in fibroblasts from SMA patients, p53 redistributes to the nucleolus. This finding suggests that the SMN protein has the ability to suppress the pro-apoptotic properties of p53 by physically sequestering p53 in a cellular compartment that inhibits p53 function (413). While the knockdown of the SMN gene results in embryonic lethality in mice (326), the absence of the SMN1 gene in SMA patients does not elicit detrimental effects because of the presence of the highly homologous SMN2 gene, which produces the full-length SMN protein in amounts that are sufficient to rescue all tissues, except the motor neuron. This notion is supported by the evidence that transgenic mice lacking the murine Smn gene, but bearing the human SMN2 gene, resemble the SMA phenotype in hu-

mans. Despite current hypotheses that attempt to explain the tissue-specific nature of SMA (246), the question of how a deficiency in a ubiquitous protein causes a motor neuron-specific disease remains unanswered.

5. Huntington's disease (HD). Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by selective neuronal loss in the striatum and the cerebral cortex, and manifested clinically by uncontrolled movements, general motor impairment, personality changes, and dementia. HD is an inheritable autosomal dominant disease that is caused by expansion of CAG repeats in exon 1 of the IT15 (huntingtin) gene. These repeats are translated into an expanded polyglutamine tract near the N-terminus of the huntingtin protein (367). Overexpression of huntingtin with CAG expansion in a rat hippocampal neuronal cell line induced JNK activation and apoptotic cell death. It has been suggested that the expansion of the polyglutamine tract in the huntingtin protein affects its interaction with pro-apoptotic factors. For instance, mutant huntingtin promotes translocation of the pro-apoptotic factors Smac/Diablo and Htra into the cytosol with subsequent reduction in levels of cytosolic IAP1 and XIAP. Levels of IAP1 and XIAP were, in fact decreased in HD brain tissues (110). Mutant huntingtin also binds more efficiently to p53 than wild-type Huntingtin, which upregulates the level of nuclear p53 and promotes its transcriptional activity in neuronal cultures and in HD mice. Furthermore, protein levels of the p53 targets, Bax and Puma, were significantly elevated by mutant huntingtin (14).

Aberrant interaction of mutant huntingtin with huntingtin interacting protein (HIP-1) may also contribute to HD pathogenesis. The interaction of HIP-1 and huntingtin decreased with increasing polyglutamine length such as in mutant huntingtin, thus contributing to a reduced ability of the cell to sequester pro apoptotic HIP-1 (155). Free HIP-1 binds HIP1 protein interactor, which forms HIPPI-HIP1 heterodimers. This heterodimer can recruit procaspase 8 into a complex of HIPPI, HIP1, and procaspase 8, and initiate the extrinsic apoptotic cascade (105). Overexpression of wild-type huntingtin, which is capable of binding HIP-1, significantly reduces HIP-1-induced apoptosis (123). It has been shown that mutant huntingtin induces apoptosis in cultures of primary rat neurons (318) and PC12 cells (199) by activating caspases. Accordingly, overexpression of wild-type huntingtin protected neuronal cells from apoptotic stimuli via specific inhibition of procaspase 9 processing (306). Caspase involvement is evidenced by the finding that the broad-spectrum caspase inhibitor ZVAD-fmk, was effective in slowing disease progression in a transgenic mouse model of HD (265).

Apart from animal models of HD, the participation of apoptosis in disease pathogenesis in humans is supported by the demonstration of caspases 1, 3, 8, and 9, and cytochrome *c* activation in the brains of HD patients (166, 365, 318). Current evidence suggests that the mitochondrion is a target in HD pathology. *In vitro* studies have demonstrated a deleterious effect of the mutant huntingtin protein on mitochondrial function, and lymphoblast mitochondria from HD patients and brain mitochondria from HD mice exhibit decreased $\Delta\Psi m$ (272). More recent evidence showed that mutant huntingtin decreases the levels of two subunits of mitochondrial complex II, resulting in decreased complex II activity in the HD striatum (25). Traditionally, the pathogenesis of HD has been viewed as the result of a toxic gain-of-function of the mutant huntingtin. However, an involvement of the wild-type huntingtin in the upregulation of transcription (423) and vesicular transport of BDNF (104), a critical prosurvival factor of striatal neurons, suggests that a loss of the beneficial activity of the huntingtin protein may, in fact be the contributor to neuronal death in HD.

The study of HD is facilitated by the availability of the yeast artificial chromosome (YAC) transgenic mice that express the full-length mutant huntingtin (htt) with 72 CAG repeats (YAC72) and exhibit early electrophysiological abnormalities. In these animals, initial neuronal cytoplasmic toxicity is followed by cleavage of htt, nuclear translocation of htt N-terminal fragments, and selective striatal neurodegeneration by 1 year of age (134). Increased NMDA receptor activity leading to mitochondrial dysfunction and initiation of the intrinsic mitochondrial apoptotic pathway and neuronal apoptosis has been demonstrated in YAC72 mice (416).

6. Neuronal apoptosis and diabetic encephalopathy

a. Diabetic encephalopathy and apoptotic involvement. Diabetic encephalopathy refers to the slowly progressive alterations in brain function and structure that occur in association with diabetes (27). While the concept of diabetic encephalopathy remains a subject of some controversy, studies have demonstrated changes in brain function in diabetic patients and diabetic animal models, such as alterations in cognition, neuropsychology, neurobehavior, electrophysiology, structure, neurochemistry, and elevated apoptotic activity (26, 202). These findings suggest that primary diabetic encephalopathy exists as a definable diabetic complication (202). In human type 2 diabetes, most of the cognitive impairment is mild to moderate, more pronounced in the elderly (267, 279, 340), and is consistent with a loss of critical function in the hippocampal region (206). Though less well studied, cognitive performance in type 1 diabetes (diabetic encephalopathy) has recently gained attention (226, 395). Impairment of cognitive function in type 1 diabetic individuals is accompanied by changes in neuronal structure and function (226, 395), and is characterized by mild to moderate slowing of mental speed and a diminished mental flexibility, without evident impairment of learning and memory (34, 314).

The mechanisms underlying diabetic encephalopathy are unclear. Experimental studies have suggested that neuronal apoptosis may play a crucial role in neuronal loss and impaired cognitive function. The involvement of hippocampal neuronal apoptosis in diabetic encephalopathy has been demonstrated in diabetic animal models (206, see Section III.B.6.c), and evidence of classical apoptosis was associated with decreased neuronal densities, and learning and cognitive deficits (338). It is noteworthy that patients suffering from neurodegenerative disorders such as AD, PD, and HD generally exhibit a higher prevalence of diabetes (308), which underscores a sentinel relationship between diabetes and neurodegeneration.

b. Contributing factors to neuronal apoptosis and diabetic encephalopathy. Impaired insulin/IGF axis and signaling. Impairment of insulin, IGF, and C-peptide, as well as oxidative stress induced by hyperglycemia in the CNS, have been thought to be critical contributing factors to diabetic encephalopathy.

Insulin, insulin receptors (IR), and its substrates are highly expressed in neurons of the hippocampus, amygdala, and certain cortical areas involved in learning and memory. An increasing body of evidence indicates an impairment of the insulin/IGF axis and signaling in AD, PD, HD, and diabetic encephalopathy. For instance, decreases in insulin, IGF, and their receptors have been documented in the hippocampus in diabetic encephalopathy and AD patients (203, 309). Experimentally, the impairment of brain insulin and IGF functions appeared to induce a condition similar to AD in an *in vivo* model of intracerebral streptozotocin (STZ), characterized by enhanced cell loss, increased β-amyloid and tau phosphorylation, and learning and cognitive impairment (194). STZ is a naturally occurring chemical agent that is toxic to the insulin-producing β cells of the pancreas, and is widely used to produce type 1 diabetes in animal models.

The insulin/IGF axis plays an important role in the regulation of β-amyloid levels and the phosphorylation of tau proteins (44). Physiological forms of β-amyloid (Aβ(1–40) and Aβ (1–42)) are competitive inhibitors of insulin binding and action (271, 402) wherein they block the autophosphorylation of the insulin receptor. Increased tau protein phosphorylation and neuronal apoptosis are correlated with increases in β-amyloid levels (138, 402), and insulin resistance has been shown to promote Aβ(1–40) and Aβ(1–42) peptide generation in the brain. Altered insulin sensitivity and signaling have been implicated as contributors to the AD casacade (138, Fig. 10) and to increased risk of AD neuropathology (133) as supported by the findings that insulin receptor substrate-2-disrupted mice, or neuronal specific depletion of insulin receptor in mice, exhibited increased tau phosphorylation (327, 328). The quantitative contribution of impaired insulin signaling to the pathogenesis of diabetic encephalopathy is unresolved. Desensitization of insulin receptors has been documented in type 2 diabetes (138, 402). As with insulin, the IGF systems (IGF-I, IGF-II) are perturbed in the CNS of diabetic patients, in the STZ-induced type 1 diabetic, and in type 2 diabetic BBZDR/Wor rats (220). Experimentally, induction of diabetes with STZ decreases IGF-II RNA levels in different regions of the brain (cerebral, cortex, medulla) and spinal cord, and are partially restored by insulin, and prevented by C-peptide (204). Moreover, the abnormalities in expressions of IGF-I, IGF-II, IGF-IR and insulin receptor occurred early (2 months) in the brain development in type 1 diabetic BB/Wor rats and persisted in 8-month old animals, preceding hippocampal neuronal apoptosis and cognitive impairment (203).

C-peptide deficiency. C-peptide is a peptide fragment that results from the processing of the insulin precursor, proinsulin to active insulin. C-peptide has been shown to exhibit insulinomimetic effects by activating insulin receptor activity (120), and its deficiency has been thought to be a contributing pathogenic factor in diabetic encephalopathy. The prevention of spatial learning and memory deficits and hippocampal neuronal loss in type 1 diabetic BB/Wor rats by C-peptide replacement therapy supports this suggestion. Furthermore, the administration of C-peptide partially corrects perturbed insulin receptor expression and IGF activity, and prevents neuronal apoptosis and the expression of Bax and caspase 3, and PARP cleavage in the hippocampal regions in type 1 diabetic BB/Wor rats (338). C-peptide has also been reported to inhibit neuronal apoptosis in human neuroblastoma SH-SY5Y cells induced by high glucose (205); in these cells, C-peptide stimulates cell proliferation and neurite outgrowth (205). Intriguingly, while apoptosis was attenuated by insulin alone, the effect of combined insulin and C-peptide treatment was additive, indicating that C-peptide acted at a ligand site that is distinct from that of insulin (402). Taken together, the evidence suggests that C-peptide deficiency plays a role in hippocampal neuronal apoptosis and diabetic encephalopathy.

Involvement of hyperglycemia stress. Clinically, the maintenance of normoglycemia is a critical factor in the delay or prevention of cognitive impairment in diabetes (172). Individuals

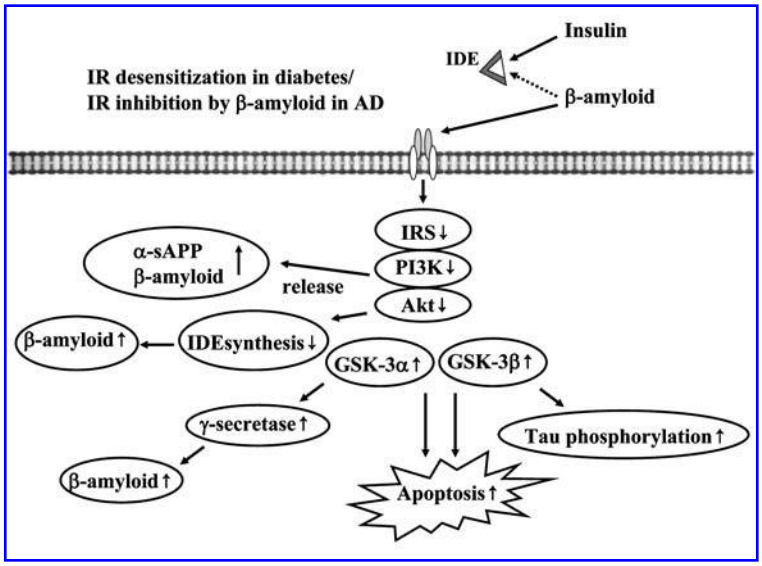

FIG. 10. Impaired brain insulin signaling and the proposed relationship between diabetes mellitus and Alzheimer's disease. The working hypothesis proposes that insulin signaling in the brain plays an important role in the reduction of β-amyloid levels and phosphorylation of tau protein. The insulin-degrading enzyme (IDE) is a metalloprotease that degrades insulin and β-amyloid. IDE favors insulin as a substrate, which leads to β-amyloid accumulation. When AD patients develop type 2 diabetes, chronically high insulin levels cause further accumulation of β-amyloid in AD through IDE activity, and this increased β-amyloid reduces the binding of insulin to its receptor. Consequently, the impairment of brain insulin signaling contributes to AD cascade in a sequlae of events: (a) impaired PI3K signaling activates the release of sAPP and β-amyloid. Since Akt controls the neosynthesis of IDE, downregulation of IDE levels leads to an increase in β-amyloid; (b) impaired PI3K/Akt signaling also leads to increases in GSK-α and GSK-β levels. Increased GSK-α enhances APP processing by enhancing γ-secretase expression, while elevated GSK-β promotes tau phosphorylation. Thus, reduced insulin signaling leads to induction of neuronal apoptosis. (Revised from Ref. 138).

with type 1 diabetes exhibit greater learning and memory impairment in association with elevated HbA1c, a marker of chronic hyperglycemia (137). Elevated glucose levels within the brain due to a dysfunctional blood–brain barrier can induce oxidative stress and promote susceptibility of the hippocampus and cortical regions of the brain. Indices of ROS-induced damage have been reported within the diabetic hippocampal regions (118). Experimentally, type 2 BBZDR/Wor rats exhibited increased hippocampal neuronal apoptosis despite normal expression of insulin receptor and C-peptide, suggesting that uncontrolled hyperglycemia *per se* is a contributor to hippocampal apoptosis. In addition, hyperglycemia enhances carbonyl stress resulting from increased formation of reactive carbonyl compound (RCOs) such as methylglyoxal (MG), a precursor of advanced glycation endproducts (245). MG-mediated oxidative stress has been shown to induce death of hippocampal neurons and human neuroblastoma cells (393). While the molecular mechanisms underlying neuronal apoptosis and diabetic encephalopathy remain unresolved, it appears that diabetes-associated perturbations in the insulin/IGF system and hyperglycemia may play prominent roles (202).

c. Animal models of type 1 and type 2 diabetes and diabetic encephalopathy. The neurological symptoms that occur in association with diabetes vary considerably between different models of diabetic encephalopathy (26). To date, a number of chemical- and gene-based type 1 diabetic (streptozotocin (STZ)-diabetic rats, BB/Wor rats, NonObese Diabetic (NOD) mice) as well as type 2 diabetic (BBZDR/Wor rats, Otsuka Long Evans Tokushima (OLETF) rats, db/db mice) rodent models are available and could prove useful for the study of diabetic encephalopathy (26). The popular rat models of type 1 diabetes (STZ-diabetic and BB/Wor rat), exhibit perturbations that are characteristic of the disease, *viz.,* loss of cognitive function, hippocampal synaptic plasticity (107, 203, 371), and complex task performance (203, 220, 296). In addition, long-term potentiation (LTP), a good indicator of the strength of synaptic transmission between hippocampal neurons, is impaired in STZ-diabetic rats (48, 107, 382). Furthermore, cognitive impairment in BB/Wor rats is associated with evidence of classical apoptosis in the hippocampus, including DNA fragmentation, positive TUNEL staining, elevated Bax/Bcl-x ratio, increased caspase 3 activities and decreased neuronal densities (203), common features in diabetic encephalopathy. Interestingly, representative type 2 diabetic rat models, such as the BBZDR/Wor rat, exhibit features of brain disturbances that are more varied in terms of behavioral and electrophysiological characteristics (26), and hippocampal neuronal loss (206). In comparison, the type 2 diabetic OLETF rat exhibits disturbances in behavior and performance on complex water/radial maze tasks as well as impairment of hippocampal LTP expression that are characteristic of the disease (204, 257).

In recent years, transgenic and knockout mouse models, such as the db/db mouse, have gained widespread appeal in diabetes research, particularly in the investigation of signaling, gene function and the mechanism of diabetes since many of the knockout models target the disruption of the major molecules in IR signaling (294). It is anticipated that these rodent models generated for classical diabetes research, will gain in popularity for future use in studies of the CNS neurodegenerative process associated with diabetes, given that diabetic encephalopathy is recognized clinically as a progressively debilitating condition that compromises the quality of life in diabetic patients.

IV. NEUROPROTECTIVE STRATEGIES

The development of neurodegenerative diseases is a process that evolves over time; this extended timeline therefore, affords an opportunity for intervention that could delay or arrest the course of the disease. In the following sections, we will review some of the therapeutic strategies that are currently in use and those that have potential for future clinical application. The discussion will focus specifically on the interventions that target neuronal cell survival; these include the use of antioxidants and inhibitors of 12-lipoxygenase expression, approaches that interfere with mediators of apoptotic death and promote activation of anti-apoptotic factors, and those that preserve mitochondrial functional integrity and inactivate caspase function. The delineation of the specific components of the apoptotic signaling pathways targeted by such interventions should provide fruitful avenues of future research.

A. Antioxidants and inhibition of 12-lipoxygenase

Antioxidants have been documented to have therapeutic value in neurodegenerative diseases. Nutritional antioxidants comprise a varied group of compounds, which have demonstrated success in experimental models and in human subjects. These include vitamin E, the green tea flavonoid, epigallocatechin gallate (EGCG), coenzyme Q, and idebenone, the mitochondria-specific antioxidant similar in structure to ubiquinone. The targets of vitamin E antioxidant action include ROS generation, pro-apoptotic Bax/Bcl-2 and p53, and anti-apoptotic HSP60 (58, 114, 358). Coenzyme Q10 targets the mitochondria and inhibits mitochondrial depolarization in rat dopaminergic neurons (248). Polyphenolic EGCG scavenges ROS and enhances PI3K/Akt survival signaling (59, 177). Idebenone, whose clinical efficacy in human AD has been validated in two large clinical trials (109, 396), attenuates β-amyloid-induced learning deficits (406).

Notable among endogenous antioxidants, is estradiol, with proven effectiveness against β-amyloid-induced neuronal apoptosis in *in vitro* models of AD and PD (100, 410). Accelerated β-amyloid plaque formation in animal models of AD is associated with brain estradiol deficiency (100). Estradiol mediates its effect by binding to the estrogen receptor, and targets a plethora of prosurvival cellular processes. These include neuronal expression of Bcl-2 members, upregulation of antioxidant proteins such as TRX, MnSOD, and nNOS, Akt signaling, and inhibition of transcriptional and apoptotic activity of the APPct complex (19, 56, 176, 410). Melatonin is another naturally occurring neuroprotectant that decreases amyloid fibril formation (274) and attenuates neuronal apoptosis in *in vitro* and animal models of AD and PD (55, 74, 239). Its neuroprotective effects appear to be the result of antioxidant and anti-amyloidogenic properties (275) and are independent of binding to membrane receptors. Given that melatonin secretion is inhibited in AD patients (243), and that production declines with age (341), par-

alleling the timeline for AD development, one could speculate that melatonin would be a suitable candidate for therapy in ameliorating or slowing the cognitive impairment in patients with AD and PD.

Finally, free radical scavengers and agents that maintain neuronal redox status have potential for use in the therapeutic treatment of neurodegenerative disorders. Carbonyl scavengers such as aminoguanidine and tenilsetam offer promise as therapeutic candidates, given that AD and diabetic encephalopathy are associated with carbonyl stress, and that carbonyl scavengers inhibit neuronal apoptosis (393). Because altered cellular redox is implicated as causative in the neurodegenerative processes associated with AD, PD, and ALS, NAC, as a reductant and a precursor of GSH, has great appeal as a neuroprotectant. In fact, NAC treatment of AD patients in a clinical trial yielded favorable results for all criteria measured, including cognitive impairment (1). Experimentally, in human neuroblastoma cells, the actions of NAC included attenuation of Aβ-induced oxidative stress, tau phosphorylation, and downregulation of APP gene transcription (352). In an animal model of AD, NAC was shown to attenuate oxidative damage, restore GSH levels, and prevent neuronal apoptosis (364).

Transcriptional antioxidant gene activation offers another approach of neuroprotection. Transcriptional activation through the antioxidant response elements (AREs) induces the production of antioxidant enzymes (324). Reportedly, the binding of the nuclear transcriptional factor, Nrf2, to AREs in gene promoters, constitutes a key regulatory factor in the coordinate induction of endogenous cytoprotective genes such as γ-glutamylcysteine ligase (γ-GCL), NAD(P)H quinone oxidoreductase 1, and heme oxygenase. These enzymes provide effective cytoprotection by regulating the intracellular redox state (324). PI3-kinase, protein kinase C (PKC), MAP kinase, and NF-κB signaling are implicated in regulating Nrf2 nuclear translocation (157, 422). Since downregulation of Nrf2 and ARE-controlled genes is involved in ALS pathogenesis (171), pharmacological agents that activate Nrf2-ARE signaling should prove effective in neuroprotection (384). We recently found that insulin affords cytoprotection against hyperglycemia-induced apoptosis in human brain endothelial cells by inducing γ-GCL expression through PI3K/Akt/mTOR signaling and Nrf2 activation (264). In other studies, activation of Nrf2-ARE has been attributed to dietary compounds like curcumin and cyclic sulphur-containing agents (189).

A promising strategy in SMA treatment is to increase the anti-apoptotic SMN protein through upregulation of SMN2 gene expression and modulation of SMN2-derived transcript splicing that favors production of the full-length SMN protein. Several compounds have been identified as possessing the ability to increase SMN levels in fibroblasts of SMA patients; early clinical trials of candidate therapeutics are now ongoing in SMA patients (240, 305).

Inhibition of 12-lipoxygenase expression. The eicosanoid (*e.g.*, arachidonate) synthesis pathway, which involves the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, is an important player in the chronic inflammatory process that contributes to neuronal loss and disease pathology in AD (18). Clinical studies have demonstrated the efficacy of COX inhibitors, but the role of LOXs in AD pathogenesis remains poorly understood. Experimentally, the 5- and 12-LOX pathways have been implicated in neuronal death mediated by kainic acid excitotoxicity (379) and oxidative glutamate toxicity (164, 201), and apoptosis induced by prion peptide (351). In recent studies, Lebeau *et al.* (188), using an antisense oligonucleotide-based approach, demonstrated that inhibition of 12-LOX expression prevented Aβ-induced apoptosis in cortical neurons in conjunction with the suppression of c-Jun expression. However, c-Jun-dependent cortical neuron apoptosis was promoted by the 12-LOX metabolite, 12(S)-hydroxy-(5Z, 10E, 14Z)-eicosatetraenoic acid (12(S)-HETE). Importantly, the 12-LOX involvement was specific and was independent of 5-LOX participation (188). These results suggest that 12-LOX mediates c-Jun-dependent cortical neuronal apoptosis, and that targeting 12-LOX could prove beneficial in the treatment of AD. In a series of other studies, Khanna *et al.* (163–165) made the interesting observation that glutamate-induced 12-LOX activity was uniquely sensitive to the natural vitamin E, α-tocotrienol, at nanomolar concentrations, but not to α-tocopherol. This finding provides the first evidence that implicates α-tocotrienol as the potent neuroprotective form of vitamin E. These investigators further demonstrated that 12-LOX deficient primary cortical neurons were resistant to glutamate toxicity (164), suggesting that targeting 12-LOX by α-tocotrienol treatment is a viable strategy in neuroprotection.

B. Preservation of mitochondrial functional integrity and inactivation of caspases

Mitochondrial preservation. Mitochondrial health is a target of multiple intervention strategies given the centrality of the mitochondrion in apoptotic signaling. Nicotinamide, a precursor for coenzyme β-nicotinamide adenine dinucleotide (NAD^+), acts directly at the mitochondrial membrane pore to prevent cytochrome *c* release (64), and reverses mitochondrial membrane depolarization and MPTP formation induced by *t*BH (65). Of relevance to neurodegeneration is the fact that AD is associated with increased mitochondrial permeability, and that β-amyloid mediates MPT pore opening that results in loss of volume regulation and mitochondrial swelling (63). Insulin and IGF-I, through activation of the PI3K/Akt cascade, promote neuronal survival by preventing the initiation of mitochondrial apoptotic signaling. Activated Akt mediates multiple survival signals; these include Bad phosphorylation, which inhibits mitochondrial Bax translocation (407); altered mitochondrial pore function independently of Bcl-xL function; and binding of mitochondrial hexokinase to the voltage-dependent anion channel of MPT pore (76), which prevents mitochondrial cytochrome *c* release and apoptosis initiation (224).

Erythropoietin (EPO) similarly modulates a broad array of cellular pathways that protect against β-amyloid toxicity in human neuroblastoma SH-SY5Y cells (63). At the mitochondria, EPO directly modulates $\Delta\Psi$m and cytochrome *c* translocation (63) which is linked to the function of Bcl-xL, maintains the expression of Bcl-2 and Bcl-xL, and alters the Bcl/Bax ratio, which favors anti-apoptosis (380). Recent studies confirm that upregulation of Bcl-xL by EPO is necessary for apoptosis prevention in cerebral endothelial cells (60). Other EPO function is dependent upon PI3K and Akt activation (17); the prevention of Akt phosphorylation blocks cellular protection by EPO (61).

AMP protein kinase activator (AICAR) and metformin (antidiabetic agent and AMP kinase activator) are two clinically relevant agents currently of interest; these could prove beneficial in targeting the neurodegenerative process in diabetic encephalopathy associated with loss of mitochondrial functional integrity. AMPK activation modulates mitochondrial hexokinase binding to the voltage dependent anion channel of MPT pore (76) and regulates MPT (385). Metformin and AICAR are protective against hyperglycemia and *t*BH-induced endothelial cell apoptosis through preservation of the MPT. Additionally, in endothelial cells, AICAR prevents the loss of $\Delta\Psi$m and caspase 3 activation, reverses Akt phosphorylation, and activates eNOS during hyperglycemic stress (142). AMPK activation also promotes insulin signaling through respective increases and decreases in tyrosine kinase and tyrosine phosphatase activities (81). It remains to be determined as to whether AMPK activators function to preserve neuronal mitochondrial integrity.

Caspase inactivation. Various caspase inhibitors prevent neuronal loss in animal models of stroke and head injury; unfortunately, to be neuroprotective, currently available caspase inhibitors must be injected directly into the brain in large quantities (51), which would be contraindicated for human application. Recently discovered endogenous inhibitors that block caspase activation belong to the IAP family such as NAIP, XIAP, and human inhibitor of apoptosis protein (HIAP) (51). Adenovirally-mediated overexpression of NAIP or XIAP was found to reduce hippocampal neuronal loss following transient forebrain ischemia. Reportedly, Smac/Diablo activates caspase 3 and promotes apoptosis by binding to and antagonizing XIAP (132); one of the mechanisms of Aβ-cytotoxicity in AD is downregulation of XIAP (361). Therefore, upregulation of IAP by gene delivery could prove to be a practical and novel therapeutic approach in the treatment of neurodegenerative disorders (101).

C. Neurotrophic factors and inhibition of neuronal apoptosis

Neurotrophic factors have emerged as major anti-apoptotic agents that target neuronal apoptosis and show promise for the treatment of neurodegenerative disorders. A comprehensive coverage of the various factors is beyond the scope of the current review; our focus will be on the more well studied anti-apoptotic mechanisms of insulin, IGF-I, and glucagon-like peptide (GLP)-1, all with proven effectiveness in attenuating neurodegenerative processes associated with AD and diabetes.

Insulin and IGF-I regulate the actions of β-amyloid and its precursor (APP), and the phosphorylation of tau (44). Importantly, IGF-I crosses the blood–CNS barrier (220) and protects hippocampal neurons against β-amyloid toxicity. Trophic factor promotion of cell survival is attributed, in part, to PI3K/Akt activation, a central anti-apoptotic pathway. Activated Akt-targeted processes include phosphorylation of mTOR and cyclic AMP response element–binding protein (CREB), phosphorylation-inactivation of Bad and caspase 9, decreased glycogen synthase kinase (GSK)-3β activity, and modulation of NF-κB and the forkhead Box class O (FOXO) members (62, 253, Fig. 11). In hippocampal neurons and PC12 cells, IGF-1/Akt signaling mediates FKHRL (a FOXO member) phosphorylation at its DNA binding site, and prevents transcriptional activation, which promotes cell survival (244, 418). Other key effects of IGF-1/Akt signaling in neuronal protection are: (a) degradation of IκB which promotes NF-κB activation and anti-apoptotic IAP expression (149), and (b) inhibition of GSK-3β activation which prevents cytochrome *c* release and caspase 3 activation (178), and APP processing and tau phosphorylation (8). More recently, we showed that insulin/Akt activation mediates mTOR/p70S6K signaling and the induction of γ-GCL, which results in *de novo* synthesis of GSH (264). Selective inhibition of mTOR induces apoptosis in multiple cell types (108). Significantly, phosphorylated forms of mTOR and p70S6K are reduced in the cortex of the APP/PS1 transgenic mice (186), and anti-apoptotic mTOR/p70S6K signaling is altered in cellular and transgenic models of AD. Indeed, Aβ-induced downregulation of mTOR/p70S6K phosphorylation in murine neuroblastoma cells is associated with caspase 3 activation.

Glucagon-like peptide (GLP)-1. Intestinal derived GLP-1 is an endogenous insulinotropic peptide that readily enters the brain (283). GLP-1 is also synthesized in select brainstem and hypothalamic neurons, and GLP-1 receptors (GLP-1R) are widely expressed in the CNS and hippocampus of rodents and humans. GLP-1 decreases brain β-amyloid levels *in vivo*, and protects hippocampal neurons against β-amyloid-induced neurodegenerative processes (283). Antagonism of GLP-1 action enhanced β-amyloid-induced apoptosis in rats (261). Additionally, GLP-1 decreased APP levels in cultured neuronal cells,

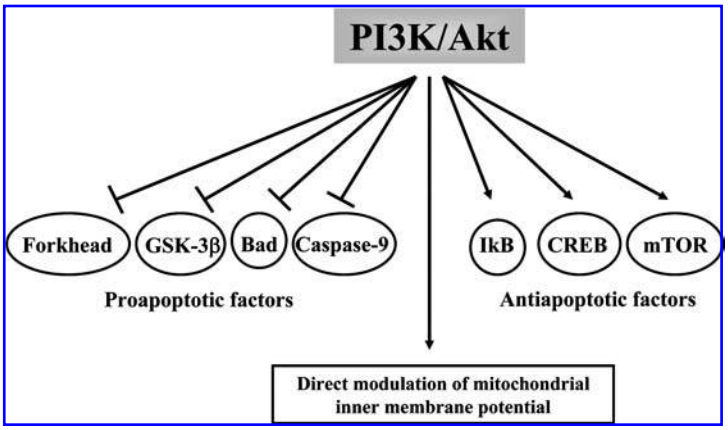

FIG. 11. Relationship between PI3K/Akt signaling and various pro- and anti-apoptotic factors. PI3K/Akt signaling has been identified as a central component in promoting cell survival and attenuating apoptosis. PI3K/Akt signaling blocks pro-apoptotic signals through regulation of the forkhead family members, GSK-3β, Bad, and caspase-9. Additionally, PI3K/Akt signaling promotes anti-apoptotic signals through IκB, CREB, and mTOR. Furthermore, Akt acts directly at the level of the mitochondrial inner membrane potential and alter mitochondrial pore function.

facilitated differentiation and induced neurite outgrowth in PC12 cells (284), and protected against apoptosis in cultured hippocampal neurons (285). Through GLP-1R signaling, GLP-1 enhances associative and spatial learning (86). Mechanistically, GLP-1 acts by binding to G-protein coupled receptor (35); ligand-mediated activation of Gα subunit of GLP-1R stimulates adenylate cyclase and intracellular cAMP, and activation of protein kinase A (35, 283). Additionally, the G$\beta\gamma$ dimer activates the PI3K/Akt and MAP kinases (35, 283) which are neuroprotective (283, 286). While GLP-1 has the potential to be a novel and attractive treatment modality for patients with AD (Fig. 12), its efficacy in neuroprotection against diabetic encephalopathy is less clear (98, Fig. 12).

Other growth factors that hold promise as potential anti-apoptotic agents in neurodegeneration are nerve growth factor (NGF), transforming growth factor-β (TGF-β), gastrointestinal peptide (GIP), fibroblast growth factor (bFGF), and brain-derived neurotrophic factor (BDGF). NGF binds two structurally different classes of cell surface receptors, *viz.,* the tyrosine kinase Trk and p75 NTR receptors, both of which are expressed on neural cells (283). NGF-Trk signaling promotes cell survival and differentiation (344), whereas NGF-p75NTR receptor binding induces apoptosis (102). The observation that TrkA receptor is downregulated in diabetic rats and AD, while p75 NTR is elevated (206) suggests a role for NGF in neuropathophysiology. Collectively, TGF-β and bFGF exhibit similar neuroprotection of cultured hippocampal neurons by attenuating β-amyloid toxicity (30, 233). Neuronal expression of the TGF-β type II receptor is decreased early in the course of AD, and attenuated TGF-β signaling reportedly increased Aβ deposition and neurodegeneration in a mouse model of AD (70). In comparison, BDNF prevents apoptosis of cultured neurons by inducing the expression of antioxidant enzymes and Bcl-2 family members (53, 283). During progression of AD, BDNF in the parietal cortex is decreased (218), and BDNF administration in conjunction with Sonic Hedgehog increases the number of cholinergic neurons in culture (304). The function of GIP is interesting; engagement of GIP receptors in the brain activates stem cell proliferation and differentiation into neurons, suggestive of a role in brain repair (258).

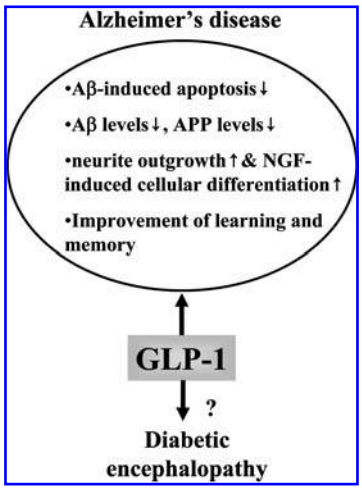

FIG. 12. GLP-1 action in Alzheimer's disease and diabetes. GLP-1 may represent an attractive therapeutic method for neurodegenerative disorders, including AD and possibly diabetic encephalopathy due to its neuroprotective effect. The neuroprotective effects of GLP-1 against AD pathology include decreased β-amyloid in the brain *in vivo*, reduced APP levels in cultured neuronal cells, and protection of hippocampal neurons against β-amyloid-induced neuronal apoptosis. GLP-1 also facilitates differentiation and induces neurite outgrowth in neuronal cells, and enhances associative and spatial learning through its GLP-1R. Despite the similarities between AD and diabetic encephalopathy in their pathogenesis, the protective effect of GLP-1 against brain neuronal apoptosis in diabetic encephalopathy is less well known.

Despite the promise of neurotrophins as potential neuroprotectants, most of the classic neurotrophic factors have, to date, not proven to be clinically useful neuroprotective agents in humans, due to peptide inaccessibility across the blood–brain barrier. The future success of trophic factors in neuroprotection against the degenerative process will hinge on the design of small molecules with growth factor potential and the ability to traverse the blood-brain barrier.

V. SUMMARY AND PERSPECTIVE

Apoptosis is a well-recognized biological phenomenon in the physiological processes of synaptogenesis, neurodevelopment, and organ involution. The explosion of apoptosis research in neurodegeneration has stemmed from the notion that induction of neuronal apoptotic death may play center stage in disease progression and that anti-apoptotic strategies could prove effective in the prevention of neurodegenerative processes. At the basic science level, major research efforts have focused on elements of the apoptotic machinery, such as the death receptor and mitochondrial apoptotic pathways, and the function of caspases and protein members of the Bcl-2 family. Two recent advances, *viz.*, our understanding of the interaction of cytochrome *c* and cardiolipin, as well as the potential role for mitochondrial redox, and the oxidative status of mitochondrial DNA in apoptosis initiation should stimulate exciting avenues for future basic research. The recent advances in the development of gene-based mutant mouse models for the study of various specific neurodegenerative diseases, has not only provided critical insights into the genetic basis and specific gene mutations in disease genesis, but will allow for the investigation of the role for oxidative stress and apoptotic involvement in neuronal demise *in vivo*. A better conceptual insight into both gene based and cellular/metabolic based factors that contribute to the neurodegenerative processes should provide the knowledge base for future design of targeted and successful therapeutic interventions and treatment modalities.

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ABBREVIATIONS

$\Delta\psi$m: mitochondrial membrane potential; 3-NP: 3-nitropropionic acid; 6-OHDA: 6-hydroxydopamine; 8-oxodG: 8-oxodeoxyguanosine; AD: Alzheimer's disease; AGEs: advanced glycation end-products; AICAR: AMP-activated protein kinase activator; AIF: apoptosis inducing factor; Akt: protein kinase B; ALPS: autoimmune lymphoprolipherative syndrome; ALS: amyotrophic lateral sclerosis; AMPK: AMP-activated protein kinase; ANT: adenine nucleotide translocator; AP: apurinic/apyrimidinic site; Apaf-1: apoptotic protease-activating factor-1; Ape1: apurinic endonuclease 1; APP: amyloid precursor protein; ARE: antioxidant response element; ASK1: apoptosis signal-regulating kinase 1; ATM: ataxia-telangiectasia-mutated protein kinase; ATR: ataxia-telangiectasia Rad3-relates protein kinase; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; BER: base excision repair; bFGF: basic fibroblast growth factor; BIR domain: Baculovirus IAP repeat domain; CARD: caspase activation and recruiting domain; cdc27: cell division cycle protein 27 homolog; CED3: bacterial caspase3; cIAP1: cellular inhibitor of apoptosis protein 1; cIAP2: cellular inhibitor of apoptosis protein 2; CNS: central nervous system; COX: cyclooxygenase; CREB: cyclic AMP response element–binding protein; CSF: cerebrospinal fluid; DAXX: death domain-associated protein; DcR1: decoy receptor 1; DcR2: decoy receptor 2; DcR3: decoy receptor 3; DD: death domain; DED: death effector domain; DFF45: DNA fragmentation factor 45 kD subunit; Diablo: direct IAP binding protein of low pI; DISC: death inducing signaling complex; DJ-1: PD-associated gene; DLK: dual leucine zipper-bearing kinase; DNA-PK: DNA-dependent protein kinase; DR4: death receptor 4; DR5: death receptor 5; EGCG: epigallocatechin gallate; endoG: endonuclease G; eNOS: endothelial NOS; EPO: erythropoietin; ER: endoplasmic reticulum; ERK: extracellular signal-regulated kinase; FAD: familial AD; FADD/TRADD: Fas- and TRAIL-associated death domain; FasL: Fas ligand; FKHRL1: forkhead homolog (rhabdomyosarcoma) like 1; FOXO: forkhead box class O; GAPDH: glyceraldehide-3-phosphate dehydrogenase; GIP: gastrointestinal peptide; GLP: glucagon-like peptide; GPX: GSH peroxidase; GRX1: glutaredoxin1; GSH: reduced glutathione; GSK: glycogen synthase kinase; GSSG: glutathione disulfide; GST: glutathione S-transferase; HD: Huntington's disease; HDM2: human double minute 2 protein; HIAP: human IAP; HIP-1: huntingtin interacting protein; HNE: 4-hydroxynonenal; HO1: heme oxygense1; HSP: heat shock protein; HtrA2: high temperature requirement A2; IAP: inhibitor of apoptosis proteins; ICAD: caspase-activated DNase; ICH1: bacterial caspase1; IDE: insulin-degrading enzyme; IGF: insulin growth factor; IKK: inhibitory κB (IκB) kinase kinase; iNOS: inducible NOS; IR: insulin receptor; IRS: insulin receptor substrate; IT15: huntingtin gene; jBid: JNK-mediated truncated Bid; JNK: c-Jun N-terminal kinase; Keap1: Kelch-like ECH-associated protein 1; LOX: lipoxygenase; LRRK2: leucine rich repeat kinase 2; LTP: long term potentiation; LZK: leucine zipper-bearing kinase; MAPKK: mitogen-activated protein kinase kinase; MEF: mouse embryonic fibroblasts; MG: methylglyoxal; MKK3/6: MAP kinase kinase 3/6; MLK: mixed lineake kinase; MnSOD: manganese superoxide dismutase, mitochondrial form; MPT: mitochondrial permeability transition; MPTP: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; MQ: menadione, 2-methyl-1,4-naphtoquinone; mtDNA: mitochondrial DNA; mtGSH: mitochondrial GSH; mTOR: molecular target of rapamycin; NAC: *N*-acetylcysteine; NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; NAIP: neuronal apoptosis inhibitory protein; NDUFS1: 75 kD subunit of respiratory complex I; NF-κB: nuclear transcription factor kappa B; NGF: nerve growth factor; NIK: NF-κB inducing kinase; NIRKO: brain/neuron-specific insulin receptor knockout mice; NK cells: natural killer cells; NMDA: *N*-methyl-D-aspartate; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; NOD mice: non-obese diabetic mice; Nrf2: nuclear factor-erythroid 2 p45-related factor 2; NRG: neuregulins; OGG1: oxoguanine DNA glycosylase; OLEFT rats: Otsuka Long Evans Takushima rats; $ONOO^-$: peroxinitrite; OPG: osteoprotegerin; $p21^{WAF1}$: cyclin-dependent kinase inhibitor 1A; $p27^{KIP1}$: cyclin-dependent kinase inhibitor 1B; Par-4: apoptosis response-4 protein; PARP: poly(ADP-ribose) polymerase; PD: Parkinson's disease; PDI: protein disulfide isomerase; PI3K: phosphatidylinositol 3-kinase; PIDD: p53-inducible protein with DD; PINK-1: PTEN-induced kinase 1; PKC: protein kinase C; PKCδ: protein kinase C, δ isoform; PS: presenilin protein; PTEN: phosphatase and tensin homolog deleted on chromosome ten; PTP: permeability transition pore; RAIDD: RIP associated ICH1/CED3 homologous protein with DD; RCO: reactive carbonyl compound; RIP1: receptor-interacting protein 1; ROS: reactive oxygen species; Siah1: seven in absentia homolog 1; SIDS: sudden infant death syndrome; SMA: spinal muscular atrophy; Smac: second mitochondria-derived activator of caspases; SMN: survival motor neuron; SN: substantia nigra; snRNPs: small nuclear ribonucleoproteins; SOD: superoxide dismutase; SOD1: Cu/Zn SOD, copper/zinc SOD, cytosolic form; STZ: streptozotocin; TAK1: transforming growth factor β-activated kinase 1; tBH: *tert*-butylhydroperoxide; tBid: truncated Bid; TGF-β: transforming growth factor-β; TNF: tumor necrosis factor; TNFR1: TNF receptor 1; TPL2: tumor progression locus 2.; TRAF2: TNF-α receptor-associated factor 2; TRAIL: TNF-related apoptosis-inducing ligand ; TRAIL-R1: TRAIL receptor 1; TRAIL-R2: TRAIL receptor 2; Trk: tyrosine kinase; TRX1: thioredoxin 1; TUNEL: terminal transferase-mediated UTP nick end-labeling; UPR: unfolded protein response; VDAC: voltage-dependent anion channel; Wee1: wee-like protein kinase; XIAP: X chromosome-encoded IAP; YAC: yeast artificial chromosome; γ-GCL: γ-glutamylcysteine ligase.

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Address reprint requests to:
Tak Yee Aw
Department of Molecular and Cellular Physiology
Louisiana University Health Sciences Center
1501 Kings Highway
Shreveport, LA 71130

E-mail: taw@lsuhsc.edu

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