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Forum Review

Oxidative Stress and Lysosomes: CNS-Related Consequences and Implications for Lysosomal Enhancement Strategies and Induction of Autophagy

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ABSTRACT

The central nervous system is notable for its level of oxygen utilization and ATP synthesis, resulting in a distinct susceptibility to oxidative stress. Generation of reactive oxygen species (ROS) can occur with mitochondrial respiration as well as during other aspects of cellular homeostasis maintained through a balance between biosynthesis and catabolism. Altered catabolic processes often promote oxidative stress, and the autophagy–lysosome pathway stands out as being both affected by and contributing to the resulting stress. ROS production is increased by aging, excitotoxicity, and aberrant protein processing, just a few of the events that also influence lysosomal degradative mechanisms. Oxidative damage leads to very different outcomes, such as compromise of lysosome integrity as well as potential compensatory responses involving amplification of lysosomal enzymes and induced autophagy. Lysosomal activation occurs with brain aging, is a characteristic feature of Alzheimer's disease, and has been suggested to be an avenue for preventing protein accumulation pathology. This review provides examples from the literature to discuss the role of lysosomes in oxidative damage, the brain's distinct vulnerability, and issues regarding the enhancement of lysosomal capacity and autophagic processes. *Antioxid. Redox Signal.* 8, 185–196.

Catabolic processes, which are involved in normal protein turnover and production of nutrients for the cell, sporadically produce chemical variants that possess reactive groups. Major degradation and recycling of cellular material occurs in either lysosomes or autophagolysosomes of the macroautophagy pathway. The organelles contain several classes of hydrolytic enzymes that mediate the digestion of cellular components, phagocytosed material, and intra- and extracellular debris, and these digestive functions are sensitive to oxidation. Oxidative stress occurs in a cell when an imbalance exists between generation and elimination of reactive oxygen species (ROS). Such free radicals can damage important structural elements necessary for cellular morphology, as well as alter the function of a multitude of enzymatic pathways that express and maintain a cell's phenotypic character. Increases in nucleic acid, lipid, and, in particular, protein oxidation are believed to be mediators of the cellular changes associated with aging (45, 60). Correspondingly, increasing resistance to oxidative stress is thought to explain the role of autophagy genes and caloric restriction in lifespan extension (70, 98, 108). Many types of cells and tissues accumulate oxidatively damaged material during aging and age-related disorders. In particular, the brain, its enormous content of neuronal connections between axons and dendrites, and their intricate mechanisms for memory encoding, are all disrupted by such oxidative stress. Thus, it is not surprising that neuronal connections have been reported to be targeted by the oxidative damage associated with excitotoxicity (25, 105), as well as with age-related disorders including Alzheimer's (63, 64) and Parkinson's disease (78).

OXIDATIVE STRESS AND NEUROPATHOGENESIS

There is a relationship between oxidative stress and the neurotoxicity that is linked to the abnormal firing of excita-

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tory neurons (i.e., excitotoxicity). The brain contains a vast array of excitatory connections, causing a propensity for excitotoxic episodes of varying degree that can feed into age-related ROS production (Fig. 1, upper left). It is believed that neurons are the most vulnerable cell type in the brain and that such vulnerability to age-related oxidation mediates brain aging (59, 60). Excitotoxicity leads to ROS production and the oxidative agents in turn promote excitotoxic mechanisms, usually by disrupting calcium homeostasis and activating calcium-dependent proteases including members of the calpain and caspase families (25, 86). Changes in intracellular calcium can occur through the abundant ionotropic glutamate receptors, the major type of transmitter receptors in the central nervous system. Aberrant proteolysis by deregulated protease activity has been implicated in a variety of neurodegenerative conditions and age-related challenges (75, 114). Such proteolysis not only contributes to oxidative damage, but may also degrade enzymes that are responsible for eliminating cellular ROS including lipid peroxides (90). Together, a self-perpetuating cycle of ROS production is apparent, consisting of excitotoxicity—aberrant proteolysis—ROS production—excitotoxicity (Fig. 1, cycle A).

Excitotoxic processes underlying such problems as stroke and cerebral ischemia are compounded by a variety of circumstances, including the abundance of glutamatergic receptors that represent the primary mediators of excitatory neurotransmission. Progressive damage in the ischemic brain may be caused by a pathological cycle that involves reversible suppression of neuronal responses via oxidative stress, followed by hyperexcitability, enhanced activation of NMDA-type glutamate receptors, and concomitant ROS generation (4, 116). In addition, the data suggest that oxidative stress facilitates the unmasking of quiescent NMDA receptors via inhibition of redox-sensitive glutamate uptake. Even brief periods of calcium influx through NMDA receptors can cause sustained calpain activation and associated neuropathology (10, 12, 93). Further complications exist as calpain activation can lead to lysosomal membrane damage (118), and the lysosomal perturbation may feed back to promote additional calpain responses that cause cytoskeletal compromise (16).

Interestingly, calpain and its aberrant proteolytic activity can target select organelles that are well studied for their involvement in age-related neurodegeneration. Besides causing lysosomal damage, calpain was found to cleave bid and bax to initiate mitochondrial dysfunction, leading to caspase-independent (27, 103) and caspase-3-associated apoptotic cell death (39). The release of cytochrome *c* that is associated with mitochondrial dysfunction possibly causes increased ROS production (21). Oxidative stress is associated with both mitochondrial and lysosomal dysfunction (30, 35). Accumulation of oxidized material within lysosomes may reduce autophagic processes and thus mitochondrial recycling, leaving dysfunctional mitochondria to remain in the cytosol (20). This constitutes the mitochondrial–lysosomal axis theory that may account for certain aspects of aging and age-related disorders via imperfect autophagocytosis. Proteasome inhibition has also been demonstrated to dramatically alter mitochondrial homeostasis and impair lysosome-mediated degradation of mitochondria, and such events are associated with increased autophagy and potentially contribute to brain aging (104). It is noteworthy that excitotoxic processes, including calpain activation, may be involved in further modes of mitochondrial changes and related pathogenesis. NMDA-type excitotoxicity affects mitochondria, resulting in apoptosis that is independent of cytochrome *c* and caspase activation (116). In addition, periodic episodes of excitotoxic activity enhance the neuronal vulnerability to cellular changes associated with aging (9, 13, 31). Together, these reports suggest that aberrant proteolysis via calpain and/or other protease families form a link between stroke-type excitotoxicity and age-related neurodegeneration.

Alzheimer's disease is a good example of an age-related disorder to point out contributions of oxidative stress. Oxidative damage is thought to be a major and perhaps causal fac-

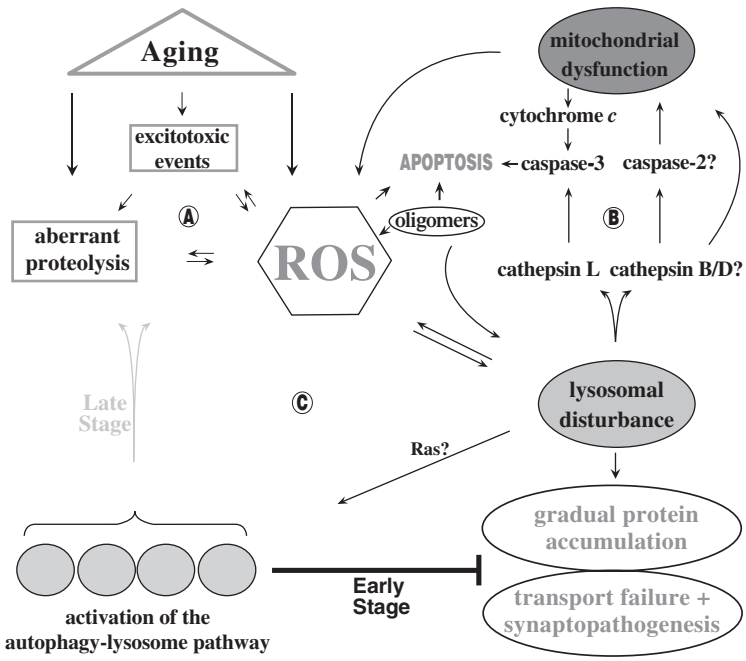

FIG. 1. Contributors of oxidative stress in the CNS. Potential pathways of positive feedback that lead to neurodegeneration include: excitotoxicity—aberrant proteolysis—ROS—excitotoxicity (**A**); ROS—lysosomal disturbance—mitochondrial dysfunction—ROS (**B**); and lysosomal disturbance—autophagic/lysosomal activation—aberrant proteolysis/ROS—lysosomal disturbance (**C**). The latter cycle is suggested to occur at late-stage oxidative stress, at which time the considerable level of lysosomal dysfunction has a negative influence on the entire autophagy–lysosome pathway. At the early stage of ROS production and lysosomal disturbance, induced autophagy and enhanced lysosomal enzyme levels have been shown to block the pathogenic cascade associated with protein accumulation/aggregation.

tor in Alzheimer's disease, promoting the aberrant processing of cellular material (46, 94, 96, 128). Amyloidogenic peptides and oligomers, generated from the amyloid precursor protein (APP), are hallmark features of the Alzheimer brain, and they have been reported to form free radical species (35, 47, 53) and to produce membrane oxidative stress (30, 35, 36, 119). The lipid oxidation attacks lysosomes and causes lipid alteration and accumulation; such accumulation may also be involved in Parkinson's disease (18). Neuronal oxidative damage appears to be a common pathogenic mechanism among the major age-related disorders: Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (46). Neurons targeted by Alzheimer-type pathogenesis are vulnerable to lipid peroxidation and nucleic acid oxidation, and resultant mitochondrial abnormalities may propagate the oxidative damage (see 94, 128). The high energy requirement of the brain puts a considerable demand on mitochondria, a principal source of energy for all eukaryotic cells (except mature erythrocytes), through oxidative phosphorylation and ATP production. Mitochondrial respiration has the potential to generate ROS, and perturbation of mitochondria greatly increases the damaging ROS production.

INVOLVEMENT OF LYSOSOMES

Lysosomes represent a major pathway necessary for protein turnover and recycling cellular ingredients throughout an animal's lifespan. Part of lysosomal function is fusion with autophagic vacuoles (autophagosomes) to form autophagolysosomes. This event by which old or damaged organelles are digested in lysosomes is called autophagy. Whereas eukaryotic processes for cellular catabolism are mediated by both proteasomal and autophagic mechanisms, only autophagy has sufficient capacity to degrade defective organelles and facilitate large-scale recycling (92). Altered proteolysis involving proteasomal and lysosomal pathways contributes to neuropathogenic episodes including protein aggregation and increased oxidation. Reduction in proteasome function puts stress on lysosome capacity and causes excessive activation of the lysosomal system (34, 104, 109). In the aging brain, lysosomes become highly susceptible to oxidative stress, leading to a gradual loss of processing capacity over a lifespan. Free radicals are generated from aberrant products of incomplete catabolism, and this exacerbates the lysosomal perturbation when the organelle membrane is subjected to oxidative damage (19, 35, 119, 127). Alternatively, aberrant proteolytic events such as those associated with aging and excitotoxicity may overload lysosomes with partially digested and oxidatively damaged material (9, 97, 100, 114). Such oxidative stress activates chaperone-mediated autophagy and the oxidized proteins exhibit increased susceptibility to be taken up by lysosomes (61), causing further lysosomal strain that can impact cell viability.

Evidence for the participation of lysosomes in cell death has increased dramatically (50, 51, 57, 82, 99, 112). Lysosomes are prone to altered catabolic chemistries for they are concentrated zones of hydrolytic breakdown processes. Aberrant breakdown products often have toxic effects either by direct chemical influence in the case of free radicals, or indirectly through alteration of lysosomal integrity that can lead to the disruption of other organelles (17, 20, 50, 126). Accordingly, the generally accepted 'lysosomal pathway of apoptosis' involves ROS production and mitochondrial changes. Oxidatively damaged lysosomes release enzymes that initiate pathogenic steps of a damaging lysosomal-mitochondrial relationship.

The pro-apoptotic caspases were often considered as sole executioners of programmed cell death, but today there are many studies indicating that lysosomal hydrolases are also involved and perhaps working in tandem with select caspases (51, 112, 113). For instance, oxidative cell death caused by the redox cycling quinone naphthazarin (82) and hydrogen peroxide (74) appears to be mediated by, at least in part, lysosomal rupture and resulting relocation of damaging hydrolases. Several types of acid hydrolases are packaged in the acidic environment of lysosomes: nucleases, glycosidases, lipases, phosphatases, phospholipases, sulfatases, and proteases. The proteases include a large collection known as the cathepsins, and these enzymes appear to be involved in pathogenic cascades. The aspartic acid protease cathepsin D exhibits a high lysosomal concentration along with the cysteine proteases cathepsins B and L. The cysteine-based hydrolases also include cathepsins C, F, H, K, O, S, V, W, and X. Another loosely related member is the serine-based cathepsin G found in a lysosome-related secretory organelle, the azurophilic granule of neutrophils.

Once released from damaged or leaky lysosomes, cathepsins can contribute to different modes of oxidative damage and cell death associated with oxidative stress. Several cathepsins have been implicated as mediators of apoptotic and proinflammatory processes, including cathepsins B, D, and L (50, 54, 55, 83, 87). Whereas oxidative stress is often linked to mitochondrial permeability transition (49), studies indicate that cathepsins B and D are released into the cytosol prior to mitochondria releasing cytochrome *c* and resultant caspase activation (17, 36, 56, 57, 82, 83, 113). Zhao *et al.* (126) reported an amplification mechanism in which early release of cathepsins from lysosomes causes the activation of phospholipase A2, which in turn was found to rupture preparations of lysosomes. Interestingly, they also reported that purified cathepsins B and D caused increases in ROS production from purified mitochondria. The current understanding then, as shown in Figure 1, is that cathepsins B and/or D may have direct and indirect effects on mitochondria to promote ROS production and apoptosis. Note that a feedback pathway is evident that involves a loop of ROS generation, lysosomal disturbance, mitochondrial dysfunction, and back to ROS generation (Fig. 1, cycle B). This feedback loop may trigger apoptosis from different points, and it can feed into the age-related feedback pathway involving excitotoxic episodes (Fig. 1, cycle A). The pathogenic relationship between excitotoxic proteases (calpains and caspases) may be similar to how cathepsins and caspases act together as part of progressive oxidative damage.

Overloading lysosomes beyond their functional capacity, along with the related decay of efficient digestive properties with age, likely contributes to the aging risk factor for neurodegenerative cascades. A consistent feature of aging is the gradual production of lysosomal residual bodies comprised of lipofuscin (62). Lipofuscin and related ceroid are complex aggregates often containing proteins, lipids, and low levels of

carbohydrates and trace metals. The material is resistant to degradation due to apparent oxidation and cross-linking in lysosomes. Secondary production of lipofuscin and ceroid within lysosomes occurs through a series of Fenton chemistries, ROS formation, and ROS-related Schiff base and Maillard reactions (20, 60, 106). As lysosomes accumulate ceroid/lipofuscin, this leads to 1) impairment of lysosomal and autophagic degradation processes, 2) sensitization of lysosomes and neurons to oxidative stress, and 3) accumulation of damaged proteins and cellular components (44, 99, 110, 125). These events may be part of a larger series of changes that result in synaptopathogenesis (Fig. 1, lower right). It is clear that excessively oxidized material and abnormal metabolic products promote extensive conformational modifications, and that lysosomes are especially involved in aggregate formation.

Similar to a possible role of lipofuscin, sequestration of potentially harmful metabolic products may also be a function of glycoproteinaceous inclusions known as corpora amylacea and analogous polyglucosan bodies. The inclusions accumulate in the CNS derived from normal aging processes, and to a greater extent in Alzheimer's disease and other neurodegenerative disorders that are linked to accumulated products of catabolism (see review in 24). Problem of overload can cause normal corpora amylacea to transform to abnormal bodies found in polyglucosan disorders, similar to how lysosomal enzyme deficiencies result in lysosomal storage diseases (7). The alternative debris disposal pathway perhaps works in parallel with the lysosome/lipofuscin system in an attempt to manage and render harmless various metabolic degradation products. Neuromelanin of the substantia nigra, an intraneuronal pigment that accumulates during aging and Parkinson's disease, may also play a protective role by inactivating free radical species or chelating redox-active transition metals (123, 124). However, just as in the case of corpora amylacea and lysosomes, overloading the chelating ability of the pigment may lead to increased production of ROS, causing the selective degeneration found among neuromelanin-containing neurons in Parkinson's disease. The proteasome is another degradation route that, when altered or dysfunctional, causes an increase in autophagy, lipofuscin, and mitochondrial ROS production (104). Perhaps related to the mitochondrial damage and macroautophagy suggested to be involved in the biogenesis of corpora amylacea, especially in the CA1 hippocampal subfield (24, 28), proteasome inhibition has been implicated in the impairment of mitochondrial turnover as well as alteration of lysosome-mediated mitochondria degradation that can contribute to age-related diseases.

Vulnerability of lysosomes to oxidative damage points to their possible role in the intracellular deposition associated with different types of age-related disorders (7). In Alzheimer-type pathogenesis, lysosomal disruption is believed to be a contributing factor in the development of amyloidogenic oligomers and other protein aggregates. Lysosomal structures may also be involved in the formation of huntingtin aggregates in Huntington's disease (58). The endosomal-lysosomal system appears to be one of the pathways by which mutant huntingtin is degraded. Mutations in α-synuclein, a major protein in Lewy bodies that is linked to early-onset Parkinson's disease, result in impairment of lysosomal hydrolysis and disruption of ubiquitin-dependent degradation (102). The neurons expressing mutant α-synuclein also exhibit synaptic alterations and autophagic cell death. While the proteasome's role is debated over studies that failed to show that α-synuclein levels are affected by proteasomal inhibition, α-synuclein was recently shown to be selectively translocated into lysosomes and degraded by autophagic processing (29, 117). Thus, compromised lysosomes in the aging brain have the potential to promote distinct types of neurodegenerative cascades.

Lysosome membrane stability also has been suggested to be a factor in the progression of protein accumulation events associated with Alzheimer's disease. Amyloidogenic oligomers are selectively internalized and sequestered within lysosomes in dissociated neurons and in vulnerable regions of hippocampal tissue (11, 120), resulting in the loss of lysosomal membrane integrity and release of cathepsin D (35, 119, 120). The initial membrane damage may be related to the free radical nature of Aβ peptides (35, 46, 53). Aβ targets select neurons of the hippocampus in a stochastic manner (11). Perhaps related to such stochastic vulnerability, differences in lysosomal membrane stability and susceptibility to oxidative stress have been demonstrated across cells of the same type (74). It was further suggested that cellular resistance to oxidative stress involves, in part, lysosomes' resistance to ROS and related membrane oxidation. Disruption of the lysosomal degradative pathway likely facilitates the accumulation of other oxidatively damaged proteins. For instance, a lysosomal enzyme has been implicated in aberrant reactions and oxidative stress associated with Alzheimer-type neurofibrillary degeneration (95). As a result, some oligomerization and aggregation events appear to localize to the lysosomal system as is the case for lysosomal storage disorders.

Not only do internalized Aβ peptides promote oxidative damage, they also cause lysosomes to produce more amyloidogenic oligomers (11, 42, 120), perhaps as part of a pathogenic cycle (7). Intracellular oligomers cause early neurodegeneration (6, 42) and disrupt lysosomal function as a potential route towards synaptic pathology (Fig. 1). Alternative processing in lysosomes may increase oligomer production, and Aβ-containing carboxyterminal fragments of APP are found in compartments consistent with lysosomes and are enhanced by lysosomal dysfunction (for reviews, see 7, 77). Thus, the lysosomal compartment is conducive to the production of oligomers and oligomer precursors that often create oxidative stress. The related lysosomal disturbances may further compromise cellular pathways that process, degrade, and remove damaged and misfolded proteins. Such compromise can increase the half-life of APP fragments made in the endosomal–lysosomal system or those generated in the endoplasmic reticulum and trans Golgi network. This may influence extracellular oligomer concentrations by downregulating Aβ degradation/clearance pathways when the carboxyterminal fragments are released from neurons.

As oligomeric species influence the progression of oxidative stress, disruption of lipid and cholesterol metabolism can occur (30, 72). Thus, besides Aβ causing lipid peroxidation and ensuing permeability in the endosomal–lysosomal system (35, 119), the peptide is also associated with lipid alterations including the overproduction of ceramide. Ceramides are lipid secondary messengers, formed as N-acylsphingosines by fatty acid attachment to the sphingosine backbone

of sphingolipids. Similar to the cycles of oxidative progression described in Figure 1, Cutler et al. (30) proposed a cycle of Aβ production/oligomerization—membrane-associated oxidative stress—altered ceramide metabolism—Aβ production/oligomerization. They also found that blocking the accumulation of ceramide and cholesterol with sphingomyelin synthesis inhibitors protects against Aβ-related pathogenesis. Abnormal sphingomyelin/ceramide metabolism leads to the buildup of ceramide in lysosomes along with lipofusion, and has been implicated in Alzheimer's disease due to ceramide's ability to physically alter membrane structure/fusion to potentially disrupt endocytic processes (30, 99). In addition, abnormalities in ceramide metabolism are implicated in Parkinson's disease (18) and ischemic stroke (121). It is of interest that a direct correlation was found between altered endosomal/lysosomal function and the accumulation of ceramide (99), and buildup of the sphingolipid metabolite within endosomal/lysosomal vesicles correlated with the activation of pro-apoptotic caspases (36). Accumulated ceramide indeed appears to mediate cell death in neurodegenerative diseases, perhaps involving caspases and/or calpains (2, 14). Together, it is apparent that early chemical alterations associated with lysosomal destabilization include marked sphingomyelin hydrolysis and ceramide accumulation.

SYNAPTIC VULNERABILITY

The specialized morphology of neurons and their synaptic excitability leave neurons at risk to a variety of insults involving oxidative damage. In the brain, protein oxidation may disrupt the synaptic maintenance pathways that require elaborate transport systems for protein/organelle turnover throughout axons and dendrites. Synaptic terminals are sensitive to oxidative insults, resulting in increased levels of active p53 in synapses where it causes mitochondrial perturbation and precedes synaptic decline (41). Such p53 induction has been previously shown to trigger apoptosis through a lysosomal–mitochondrial pathway that is initiated by lysosomal membrane destabilization (122). Oxidative stress also increases calcium channel function (27) that can activate the calcium-dependent protease calpain upstream of p53. Calpain is part of excitotoxic mechanisms linked to the overstimulation of synaptic glutamate receptors. Sensitivity to p53 and oxidative damage has been shown to involve the upregulation of calpain, a key mediator of p53 induction resulting in mitochondrial membrane depolarization and caspase-dependent apoptosis (26, 88).

Certain types of oxidative damage are localized to synaptic terminals. Rats subjected to oxidative stress exhibited increased levels of lipid hydroperoxides and protein carbonyls in synaptic plasma membranes (38, 63). In addition, the oxidative damage to synaptic structures in cortex and hippocampus was implicated in the observed cognitive impairment. Disruption of distinct cytoskeletal chemistries thought to underlie functional plasticity would impact synaptic processes that govern memory encoding. Promoters of an important set of genes for synaptic plasticity and vesicular transport were selectively damaged by oxidative stress in cultured neurons, and, via transcriptional profiling, a similar set of synaptic genes exhibited reduced expression with age in the human frontal cortex (66). Thus, synaptic alterations resulting from oxidative stress are possible contributors to brain aging and age-related memory impairment.

Age-related protein conformation disorders such as Alzheimer's disease and Parkinson's disease appear to have an oxidative stress component that can have a negative impact on synaptic function (63, 78). Data from Alzheimer tissue and experimental models clearly indicate a link between lysosomal disturbances and protein accumulation that leads to synaptopathogenesis (7). Early lysosomal dysfunction by ROS and/or oligomeric species is followed by synaptic compromise. As shown in the lower right of Figure 1, synaptic perturbation is thought to result from the disruption of microtubule integrity and microtubule-based transport functions, as suggested for Alzheimer-type synaptic pathology (16, 52). Altered lysosomal function is suspected to take part in the development of both amyloidogenic material and tau deposits related to neurofibrillary tangles (reviewed in 7). Intracellular filamentous deposits of tau occur in a variety of neurodegenerative tauopathies and can also take the form of argyrophilic grains located in the temporal lobe as well as cortical and subcortical areas (43, 68). The argyrophilic grains consist of aggregates of 64- and 69-kDa tau isoforms, the same isoforms found concentrated in brain regions that are targeted by Alzheimer's disease (8). Induction of protein deposits through lysosomal dysfunction promotes distinct signs of neurodegeneration consisting of cytoskeletal deterioration, dystrophic neurites, as well as synaptic pathology including the loss of pre- and postsynaptic markers (5, 6, 9, 11, 15, 16, 22, 48, 99, 107). Synaptic markers are reduced at the protein and message levels, and such reductions correspond with protein deposition and transport failure (9, 15, 16). The reports also show that the functional integrity of synapses and distinct electrophysiological responses are disrupted during periods of lysosomal dysfunction.

Synaptopathogenesis is also a characteristic effect of oligomeric species that deposit in Alzheimer's disease. As previously discussed, oxidative stress from APP processing influences the lysosomal proteolytic pathway in such a way that promotes intracellular deposition. Related amyloidogenic peptides have in fact been implicated in distinct synaptic pathology and cognitive impairment (for a review, see 6). Fibrillar Aβ deposition also causes synaptic abnormalities and marked loss of synaptic proteins (48, 111). The synaptic vulnerability to ROS seems to be involved since Aβ1–40 caused severe oxidative modifications to proteins and lipids in synaptosomes (63). As found in the Alzheimer brain, in a transgenic mouse model, and in the hippocampal slice model, amyloidogenic oligomers can accumulate intracellularly at synapses and cause disruption of synaptic composition and function (11, 15, 107). It is curious that Aβ1–42 is internalized by neurons targeted by Alzheimer's disease and sequestered in lysosomes, causing enhanced levels of oligomer production and oxidative damage (11, 35, 119, 120). The oxidation of lysosomal membranes and associated leakage of hydrolases may promote cycling pathways of ROS generation as noted in Figure 1.

As discussed, lysosomal disturbances have been implicated in protein accumulation disorders that exhibit distinct synaptic decline and cognitive impairment. Different types of Aβ-related lysosomal perturbation as well as levels of oxidative

damage are evident among the genetic variants of ApoE and presenilin that influence Alzheimer's disease (23, 63). Other age-related disorders are likely influenced by the lysosomal instability, indeed a slow forming process in neurons that is a distinct feature of brain aging (19, 62, 73). Such gradual destabilization of catabolic pathways increases the risk for protein accumulations and aggregated protein stress responses. Coinciding with the lysosomal changes is the slow, age-related synaptic loss (67), perhaps due to synapses being particularly sensitive to oxidative stress and related lysosomotropic and mitochondrial consequences. A key reactant that is abundant in mitochondria, hydrogen peroxide, produces radicals by reacting with iron through the Fenton reaction, and causes a reduction in synaptic transmission (4). Such redox-active iron is associated with vulnerable neurons, implicating a role for iron misregulation in brain aging and Alzheimer's disease (123). Biochemical and morphological parameters indicate that synapses are the initial target in Alzheimer brains, and that synaptic decline correlates with the degree of memory impairment (6, 89). Synaptic vulnerability leaves the brain susceptible to behavioral changes and progressive mental deterioration. Synapse destabilization and reduced cerebral activity occurs with normal brain aging, and exacerbation of the synaptic changes is thought to be an early characteristic of age-related disorders. Recent focus is on synapses and synaptic maintenance with regard to understanding the influence of toxic protein and oligomeric species.

CAUTIOUS PROTECTION THROUGH LYSOSOMAL ENHANCEMENT AND INDUCTION OF AUTOPHAGY

Disturbances in lysosomes and autophagic processing that are associated with age-related disorders, lysosomal storage diseases, and oxidative stress, also are believed to contribute to synaptic pathology and cognitive decline. Much evidence links the disturbances to the progressive accumulation of such material as sphingolipids, mucopolysaccharides, proteins, and protein fragments (Fig. 1, lower right). Note that the proposed feedback cycles of oxidative progression (Figs. 1A and 1B) can feed into the toxic accumulation events and associated synaptopathogenesis. In addition to compromised degradative systems that lose their ability to digest delivered substrates, signs of induced autophagy and lysosomal upregulation are evident in diseased brains and model systems, resulting in pronounced assemblies of lysosomes and autophagic vesicles (6, 76, 92). As illustrated in Figure 1, proliferation of degradative organelles and enzymes during such 'activation' could very well promote further pathogenesis when oxidative stress reaches a sufficient stage so as to also affect the newly generated structures. Leakage of enzymes from the increased number of organelles, for instance, may lead to damaging proteolysis in the cytosol. This is depicted as a feedback of lysosomal disturbance—autophagic/lysosomal activation—aberrant proteolysis/ROS production—lysosomal disturbance (Fig. 1, cycle C). While such pathogenic feedback is proposed at *late-stage* oxidative stress, *early-stage* activation of autophagy and lysosomal enhancement have been shown in more recent studies to be protective (6). Perhaps compensatory systems are activated in response to cellular accumulations in order to promote removal of the nondigested material.

Early activation of lysosomes is a plausible compensatory response for enhancing lysosomal capacity, although the endosomal–lysosomal system has been implicated in the pathophysiology of brain aging and age-related disorders (reviewed in 7, 76, 77). Recent studies have described increases in lysosomal hydrolase activity induced in rodent and human cells, and such enhancement may be in response to the accumulation of proteins containing oxidized amino acids (15, 85). Such proteins are less readily digested, and likely facilitate protein aggregation events. Interestingly, lysosomes are also part of an established repair mechanism for resealing damaged cellular membranes (69). The lysosomal activation response increases the number of lysosomes before the onset of dementia in patients with early-stage Alzheimer's disease. Knowing the link between lysosomal dysfunction and selective neurodegeneration, a logical step toward therapeutic intervention is the enhancement of degradative processing in lysosomes. Age-related protein conformation disorders and lysosomal storage diseases may in fact be slowed or reversed by the positive modulation of the lysosomal system. Analogous treatments for the storage diseases have been proposed or developed (some of which may also help age-related disorders), including i) enzyme replacement therapies using recombinant lysosomal enzymes (33), ii) enhancement of lysosomal capacity using modulators that promote expression/trafficking/function of acid hydrolases (15, 33), and iii) modified neural progenitor cells (91). Recently, enhancing lysosomal function has been proposed as a therapeutic strategy for age-related disorders like Alzheimer's disease and Parkinson's disease (6, 7, 15, 32, 64).

As proof-of-principle for neuroprotection through lysosomal enhancement, the hippocampal slice model was treated with chloroquine to disrupt lysosomal pathways and promote protein accumulation/aggregation. Chloroquine dissipates the lysosome proton gradient required by pH-dependent hydrolases, and it also initiates a modest degree of lysosomal activation as indicated by increased hydrolase levels. This level of lysosomal activation, however, is insufficient to prevent the pathogenic cascade that drives protein accumulation and synaptic decline (5). As a means to enhance the lysosomal response in hippocampal slices, low concentrations of selective inhibitors targeting cathepsins B, D, and L trigger a feedback response that greatly enhanced protein and message levels for these cathepsins and other lysosomal enzymes, without affecting the proteasome system (15). The lysosomal modulators allowed for a controlled enhancement of the lysosomal system over a period of days to weeks that was 2–8-fold higher than the activation produced in response to chloroquine-mediated lysosomal stress. Note that lysosomal activation also occurs during ovulation where cathepsin L is upregulated (84), and with 17β-estradiol hormone treatment that induces the expression of cathepsin D (115); 17β-estradiol is known to reduce Aβ production and decrease the risk for Alzheimer's disease in postmenopausal women. In the brain, lysosomal activation may be in response to cell signaling pathways involving Ras. For instance, glycohydrolase upregulation correlates with the upregulation of the constitutively

active form of Ras (37), and both events are early markers of Alzheimer's disease (1, 22, 40).

Lysosomal enhancement in the slice model was in fact found to offset pathogenesis. First, increases in hydrolase levels corresponded with reductions in tau isoforms and amyloidogenic fragments (15). Thus, enhancement of lysosomal function appears to promote clearance of tau species that form paired helical filaments (PHF), the same isoforms found to accumulate in aged and Alzheimer brains, causing potential transport problems and enhanced oxidative stress (8, 16, 101). Second, stabilization of microtubules also was evident. Chloroquine-treated hippocampal slices that exhibit immunopositive PHF-tau and Aβ-containing fragments, displayed a clear link between the intracellular deposits and collapse of microtubule organization (16). Lysosomal enhancement restored microtubule integrity as indicated by i) reduction in tubulin breakdown, ii) increases in acetylated tubulin, a marker for stable microtubules, and iii) re-established transport mechanisms throughout axons and distal dendritic branches (15). Third and most important, such recovery of microtubule functions was critical for synapse maintenance. The protective effects of lysosomal enhancement on microtubule-based transport were coupled with restored expression of synaptic components. Therefore, positive modulation of lysosomal degradative pathways appears to reverse the pathogenic consequences ascribed to oxidative stress and intracellular deposition, including microtubule destabilization, transport failure, and synaptic compromise.

Enhancement of acid hydrolases, including several cathepsins (B, D, L, and S) and β-glucuronidase, occurs in the slice model of protein accumulation, thereby suggesting a role in a coping response to intracellular aggregates and synaptic decline. Similarly, β-glucuronidase exhibits greatly increased expression levels in Aβ-resistant neuronal cell lines, as do two other components of the endosomal-lysosomal system, arylsulfatase B and the insulin-like growth factor-II/mannose-6-phosphate receptor (65). Arylsulfatase B and cathepsins B, D, L, and S were also found to be inducible by Aβ or oxidative stress (15, 65, 85). A major function of the insulin-like growth factor-II/mannose-6-phosphate receptor is the transport of mannose-6-phosphate-containing enzymes from the trans-Golgi network to late-endosomes for subsequent trafficking to lysosomes. Together, these findings suggest that induction of endosomal–lysosomal machinery is part of compensatory signaling to protect against the pathogenic buildup of intracellular aggregates, particularly those related to Alzheimer's disease.

Mutant α-synuclein of Parkinson's disease has been suggested to be degraded by lysosomal enzymes of autophagic pathways. Both oxidative stress and mutant α-synuclein activate autophagy (61, 102), the latter causing massive buildup of autophagic-vesicular structures. Such autophagic activation has been observed in many protein conformation disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (92). Similar to events found associated with Alzheimer-type accumulations, evidence of lysosomal activation was found in cells expressing α-synuclein (71). Enhancement of lysosomal function has been suggested as a clearance mechanism for oligomeric intermediates of α-synuclein, to prevent α-synuclein-mediated cell death and perhaps slow or prevent Parkinson's and other Lewy body diseases (64). However, the lysosomal degradation pathway did not provide clearance once the intermediate oligomers formed fibrillar inclusion bodies, indicating the importance for early potentiation of clearance mechanisms (as pointed out in Fig. 1).

Early compensatory mechanisms are needed for rapid removal of protein aggregates before they can exert their toxic actions. Recent studies have reported increased clearance of α-synuclein (117) and mutant huntingtin (79, 81) when autophagy is stimulated. Interestingly, initial huntingtin expression stimulates autophagy as well as endosomal-lysosomal activity (58). Accordingly, stimulators of the autophagy–lysosome pathway have been proposed as potential therapeutics. Huntington's disease is an inherited neurodegenerative disorder caused by expanded polyglutamine proteins that accumulate abnormally as intracellular aggregates. Experiments using upstream modifiers of autophagy regulation or with rapamycin, a drug that stimulates autophagy, found increased clearance of huntingtin accumulations and other aggregate-prone proteins, as well as reduced polyglutamine toxicity (79–81). The protective results occurred in correspondence with the increase in autophagy, while inhibition of the autophagy–lysosome pathway produced opposite effects. The enhanced autophagy also protected against neurodegeneration in a fly model of Huntington's disease, and improved behavioral scores and decreased aggregate formation in a mouse model of the disease. Note that induction of inclusion body formation in response to mutant huntingtin also improved neuronal survival and reduced levels of the mutant protein (3). Thus, the autophagy/lysosomal arm of the protein degradation system and perhaps certain inclusion bodies have the ability to remove aggregate-prone material and prevent toxicity.

SUMMARY

Lysosomes represent a major degradative pathway that supports autophagic processes and responds to cell stress. They play a role in ROS production as part of a damaging lysosomal–mitochondrial connection. Oxidative damage leads to the release of lysosomal hydrolases that in turn contribute to apoptotic cascades upstream of mitochondria. Altered degradative systems likely contribute to many potential oxidation feedback cycles, which include 1) excitotoxicity—aberrant proteolysis—ROS production—excitotoxicity, 2) ROS production—lysosomal disturbance—mitochondrial dysfunction—ROS production, and 3) lysosomal disturbance—autophagic/lysosomal activation—aberrant proteolysis/ROS production—lysosomal disturbance. Such pathogenic cascades are linked to a variety of disease states including protein conformation disorders that upset brain function. The brain is extremely sensitive to oxidative stress, resulting in early signs of damage as expressed by behavioral and cognitive changes. For example, oxidative DNA damage may reduce the expression of select genes needed for synaptic communication and plasticity, and such reductions would affect memory functions and behavior. The relationship between lysosomal dysfunction and synapse maintenance also indicates that oxidative alterations can contribute to cognitive impairment and progressive neurodegeneration through de-

cline in synaptic integrity. Endogenous compensatory signals aimed to promote clearance of accumulating material appear to be activated to offset early pathology, possibly masking and delaying the onset of clinical symptoms. However, later during the course of the pathology and contributing oxidation events, compensatory responses may cause harm by promoting oxidative feedback cycles. Thus, enhancing repair responses to restore synaptic integrity and brain function, through autophagy/lysosomal modulation for instance, will always require critical consideration of the pathogenic stage at which treatment is administered.

ABBREVIATIONS

APP, amyloid precursor protein; PHF, paired helical filaments; ROS, reactive oxygen species.

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