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# Forum Review

# Proteasome Regulation of Oxidative Stress in Aging and Age-Related Diseases of the CNS

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## ABSTRACT

**Proteasome-mediated protein degradation is responsible for a large percentage of bulk protein turnover, particularly the degradation of short-lived and oxidized proteins. Increasing evidence suggests that proteasome inhibition occurs during the aging of the central nervous system (CNS), and in a variety of age-related disorders of the CNS. The focus of this review is to discuss the role of the proteasome as a regulator of oxidative stress, with preservation of proteasome function playing an important role in preventing oxidative stress, and proteasome inhibition playing an important role as a mediator of oxidative stress. In particular, this review will describe experimental evidence that proteasome inhibition is sufficient to induce mitochondrial dysfunction, increase reactive oxygen species generation, elevate RNA and DNA oxidation, and promote protein oxidation. Taken together, these data indicate that the proteasome is an important regulator of oxidative damage in the CNS, and suggest that proteasome inhibition may serve as an important switch for the induction of oxidative stress in the CNS. Additionally we discuss the likelihood that the 20S proteasome and 26S proteasome may play different roles in regulating oxidative stress and neurotoxicity in the aging CNS, and in age-related disorders of the CNS.** *Antioxid. Redox Signal.* 8: 163–172.

## FORMS OF OXIDATIVE DAMAGE IN THE CENTRAL NERVOUS SYSTEM

### *Oxidative stress*

All cells produce reactive oxygen species (ROS) as a natural byproduct of routine enzymatic and nonenzymatic processes (4, 20, 42, 52, 86). While it is clear that ROS are usually cleared from the cellular environment soon after they are produced in order to prevent ROS-induced damage, it is clear that some amount of ROS-induced damage occurs as normal part of cellular homeostasis, with limited or restricted oxidative damage apparently benign. Numerous studies have now firmly demonstrated that large-scale oxidative damage to macromolecules occurs in the aging CNS, and appears to promote a variety of adverse effects. For example, ROS may result in the inactivation of cellular factors, promote potentially deleterious "gain of function" events, or cause the generation of potentially toxic products. Once the accrual of oxidative damage reaches a point that it is no longer benign, cells are in a state commonly referred to as oxidative stress. It is important to point out that the accrual of oxidative damage, and induction of oxidative stress, is the direct result of alterations in steady state kinetics. For example, increased oxidative damage can arise as the result of either the increased synthesis of oxidative damage, impaired clearance of oxidized macromolecules, impaired repair of oxidized molecules, or reduced synthesis of new macromolecules. Increasing evidence suggests that the proteasome, a large intracellular protease, may play an important role in mediating oxidative damage that occurs during normal aging, and in age-related disorders of the central nervous system (CNS). Of considerable importance at the present time is developing an understanding as to how the proteasome is a target of oxidative stress, or whether proteasome inhibition is a mediator of oxidative stress in the CNS. In this review we provide evidence that these viewpoints are

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not mutually exclusive, and support a role for the proteasome serving as a trigger that mediates the increase in oxidative stress, neuropathology, and neurotoxicity observed in the aging CNS and age-related diseases of the CNS.

### *Oxidative damage in the CNS*

Oxidative damage is implicated as mediator of pathological and physiological disturbances observed in normal aging and age-related disease. Most notably, in 1954 Harman proposed the free radical theory of aging (4, 52), which attempted to explain cellular aging as a phenomena that is based on a single common process, namely an increased initiation of free radical reactions. The free radical theory of aging is supported by numerous observations including the fact that the accrual of oxidative damage is a general feature of aging in most cell types, with increases in oxidative damage often preceding age-related cellular dysfunction (4, 52). Additionally, some significant inverse relationships between the level of oxidation and lifespan have been reported, with increased antioxidant capacity increasing lifespan of many organisms (4, 52). Within the CNS the principle forms of oxidative damage include lipid oxidation, protein oxidation, and DNA oxidation.

### *Lipid oxidation*

All cells contain numerous forms of lipid that are all subject to exposure to ROS, and hence are subject to free radical-initiated oxidation (33, 92). Once oxidized, these lipids can form at least three different families of lipid oxidation products: 2-alkenals, 4-hydroxy-2-alkenals, and ketoaldehydes. The ketoaldehydes are the most abundant and least reactive form of lipid oxidation product, with malondialdehyde (MDA) one of the most established and well characterized ketoaldehydes (33, 92). Like the 2-alkenals, ketoaldehydes are believed to contribute little to cellular homeostasis. The 4-hydroxy-2-alkenals are highly reactive, and are the best characterized of the lipid oxidation products. In this group 4-hydroxy-2-alkenal (HNE) is the most studied. HNE induces cytotoxicity through its propensity to form Michael adducts with cysteine, histidine, and lysine residues (33, 75, 92). Because of the cytotoxic effects of HNE, and the fact that HNE is increased during aging and age-related diseases of the CNS (74, 99), numerous studies have suggested that lipid peroxidation plays a causal role in aging and age-related diseases of the CNS (33, 92). The proteasome is particularly vulnerable to HNE induced inactivation (8, 14, 34, 55, 59, 60, 73), with proteins cross-linked by HNE also serving as potent inhibitors of proteasome function (9, 37). Together, these data raise the possibility that modulation of proteasome function may be a principle mechanism by which HNE induces oxidative stress and cytotoxicity in the aging CNS. In this scenario, cells are able to withstand the generation of HNE and HNE modified proteins until the point at which they begin to inhibit proteasome function. Once the function of the proteasome is compromised, there is a switch from increased oxidative damage, to an induction of oxidative stress.

### *Protein oxidation*

The direct oxidation of lysine, arginine, proline, and threonine residues promotes the formation protein carbonyls (4, 20, 48, 52, 86). Protein carbonyls can be formed by multiple ROS-associated processes, are extremely stable, and are easily detected using commercially available assays (4, 20, 48, 52, 86). Protein oxidation increases as a function of age in multiple mammalian tissues, including the aging of the CNS (4, 20, 48, 52, 86, 92). Formation of a protein carbonyl can dramatically alter the tertiary structure of a protein, resulting in its partial or complete unfolding (4, 20, 48, 52, 86). The unfolding of proteins from their stable conformation results in the elevation of protein hydrophobicity, which confers a strong propensity for the protein to form potentially deleterious protein–protein interactions (4, 20, 48, 52, 86). Interestingly, in age-related diseases of the CNS there is an exacerbation of normal age-related increases in protein oxidation (19, 66, 74, 85). Whether this exacerbation represents an increase in protein oxidation through normal aging associated pathways, or represents the establishment of a new mechanism for generating protein oxidation, remains to be elucidated. Proteasome inhibition can contribute to elevations in protein oxidation directly, with protein oxidation occurring as the result of decreased clearance. Alternatively, proteasome inhibition can increase protein oxidation as the results of several indirect mechanisms including an elevation of ROS production and increased oxidation of nucleic acids (27). These raise the possibility that increases in protein oxidation during aging and age-related disease may be mediated in a large part through the onset of proteasome inhibition.

### *Nucleic acid oxidation*

Nuclear DNA is continually undergoing oxidative modification, which often leads to the presence of both damaged DNA bases and DNA strand breaks, with over 100 different forms of oxidative modification reported to occur to nuclear DNA (3, 6, 49), although most studies have focused solely on the 8-hydroxy-2'-deoxyguanosine (8-OHdG) lesion. Cells are equipped with a number of defense mechanisms aimed at removing and/or replacing the damaged nucleotides. Despite such efforts it appears that increases in DNA oxidation occurs during normal aging (3, 6, 50, 51), particularly the aging of postmitotic cells. In a variety of age-related neurodegenerative conditions the amount of DNA oxidation is greater than can be accounted for by normal aging (34, 66, 67, 80). In addition to DNA oxidation, it is becoming clear that RNA oxidation is another important form of oxidative damage. RNA oxidation has been reported to occur in primary CNS cultures following age-related stress, and occur in a wide variety of age-related neurodegenerative conditions (27, 71, 72, 82). It is increasingly clear that the proteasome, and proteasome inhibition play a role in regulating both DNA and RNA oxidation. Whether this is mediated as the result of enhanced ROS formation, decreased repair, or via an alternative mechanism are discussed below.

## THE PROTEASOME PROTEOLYTIC PATHWAY IN THE CNS

In order to maintain homeostasis all cells must continually degrade proteins in an efficient and reliable manner. The proteasomal and lysosomal proteolytic pathways are the two principle mechanisms by which all intracellular proteins are

degraded. Proteasomal-mediated protein degradation can be fully distinguished from lysosomal mediated proteolysis by the fact that it occurs at neutral pH, preferentially degrades short-lived proteins, occurs in a protein complex, does not involve intracellular compartmentalization, and generates peptides instead of breaking proteins down to the amino acid level. The proteasome is a large multicatalytic protease that is present in all cells (16, 21, 22, 41, 90, 100). The proteasome complex can exist as a 20S core proteasome, or consist of a 20S proteasome complex that contains two additional cap-like structures, which is referred to as the 26S proteasome (16, 21, 22, 41, 90, 100). It is important to point out that there are always far more 20S proteasome complexes than 26S proteasome complexes present at any one time in cells, and that these two different complexes have very different protein substrate specificities that are important for the CNS.

The 20S proteasome is a barrel-shaped structure, which is composed of four rings, with each ring composed of seven individual subunits. The two outer rings of the 20S proteasome are made up of alpha-20S proteasome subunits, and the two inner rings composed of beta-20S proteasome subunits. The alpha-20S proteasome subunits mediate proteasome stability, and provide a platform for the binding of the cap-like structures necessary for the formation of the 26S proteasome complex. The alpha-20S proteasome subunits do not mediate proteolysis, and therefore the beta-20S proteasome subunits are responsible for mediating all proteasome activity. Because proteins must enter at the interface between the alpha-20S proteasome subunits and the beta-20S subunits in order to be degraded (16, 21, 22, 41, 90, 100), the alpha-20S subunits do play an important indirect role in regulating proteasome activity. It is important to note that individual beta-20S proteasome subunits are responsible for mediating the different proteolytic specificities of the proteasome, with the three best characterized proteolytic activities of the proteasome being the chymotrypsin-like, trypsin-like, and post-glutamyl peptidase activities. The chymotrypsin-like activity cleaves proteins at hydrophobic amino acids, the trypsin-like proteasome cleaves at the carboxyl side of basic amino acids, and the post-glutamyl peptidase activity cleaving at acidic amino acids. Increasing evidence suggests that the expression of individual 20S proteasome subunits can be altered in response to multiple environmental and genetic stimuli (29, 58). For example, our laboratory has demonstrated for the first time that proteasome expression in neural cells is dramatically altered in response to oxidative stress (28), with other investigators demonstrating the ability of inflammatory stimuli to alter both proteasome expression and proteasome composition (45, 46).

The 26S proteasome is composed of a 20S core proteasome complex and two additional cap-like structures. In mammalian cells two different types of cap-like structures are known to exist, one defined as the PA700 or 19S complex, and the other named the 11S complex (39, 40, 53, 95). These cap-like structures bind to the outer alpha-subunits of the 20S proteasome, and form a dumbbell-like structure. These cap-like structures do not appear to mediate proteolysis, but rather appear to facilitate 20S proteasome mediated protein degradation. For example, these cap-like structures contain motifs useful in recruiting heat shock proteins and ubiquintated proteins, and also contain AAA-type ATPases that are useful in protein unfolding. Taken together, these data indicate an important role for the cap-like structures serving to increase protein substrate recruitment, and facilitate the insertion of proteins into the 20S proteasome.

The formation of the 20S proteasome is an extremely complex process, which constitutes a large amount of the total energy expenditure by cells. Consider that a relatively large portion of the total cellular proteins (up to 1–2% in certain cells, such as dividing cells) is composed of proteasome subunits, and that these subunits are sequentially brought together with the aid of various chaperones and proteoassemblin to continually form new proteasome complexes (43, 61, 81). Presumably this effort is necessary to replace damaged proteasome complexes, generate proteasome complexes with compositions that are maximized for the different cellular conditions, or to increase proteasome capacity during periods of stress. The immunoproteasome is a proteasome that contains three specialized β-subunits (LMP2, LMP7, and LMP10). The immunoproteasome has been best characterized for its role in MHC generation, but studies now indicate that the immunoproteasome also appears to be generated in neurons in response to oxidative stress and polyglutamine expression (23, 28, 30). The immunoproteasome is more resistant to oxidative stressors, suggesting that immunoproteasome formation may be important in preserving proteasome function in the face of oxidative stressors (2). Consistent with this hypothesis, neural cells with increase immunoproteasome expression exhibit a preservation of proteasome function in the face of oxidative stressors and polyglutamine expression (28, 30). However, it appears that while this may be beneficial in the short term, it may not be so beneficial in the long term. For example, neural cells with higher LMP2 expression exhibit a proteasome that is unable to respond to subsequent stressors (heat shock) (30).

## "ANTIOXIDANT" AND PRO-SURVIVAL FEATURES OF THE PROTEASOME PROTEOLYTIC PATHWAY

As we have discussed previously, proteasome-mediated protein degradation is a primary means by which oxidized proteins are degraded in cells. The degradation of oxidized proteins is ubiquitin independent and mediated by the 20S proteasome (21, 83), with proteins presumably targeted to the 20S proteasome based on their increased hydrophobicity. Mild oxidation of proteins is known to dramatically decrease their half-life and increase the rate at which they are degraded by the proteasome. This clearance of oxidized proteins is an important means by which cells are able to prevent the increase in oxidative damage (most notably increased protein oxidation), and thus proteasome-mediated protein degradation is an important "antioxidant" (47). In this capacity the proteasome aids in preventing the elevation in oxidative damage and induction of oxidative stress. This "antioxidant" feature of the 20S proteasome is not only important in the aging of the CNS, but also is likely important in numerous age-related disorders of the CNS.

Misfolded proteins can occur as the result of a number of environmental and genetic factors. In the CNS, mutations in α-synuclein, tau, Cu/Zn-superoxide dismutase, and huntingtin appear to dramatically increase their propensity for protein

misfolding and protein aggregation (1, 79). These mutations are linked to the familial and early onset of a variety of age-related neurodegenerative disorders including Parkinson's disease (PD), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Efficient removal of these mutant proteins is likely a critical factor in preventing each of these proteins from initiating processes that are deleterious to neural viability. The lysosomal pathway may play a role in mediating the degradation of some or all of these proteins (17, 64), although previous studies have supported a role for the ubiquitin-26S proteasome as mediators of their degradation. Compromises in 26S proteasome function could therefore compromise the turnover of these mutant proteins and promote neurodegeneration by allowing each of these proteins to persist in the intracellular environment for prolonged periods of time. It is interesting to note that mutations in Parkin, which is a member of the ubiquitin ligase family, are also associated with early-onset and familial PD (15, 91). Mutations in Parkin would be expected to selectively affect 26S proteasome function, which is ubiquitin dependent, and not have any direct effect on the 20S proteasome. Taken together, these data suggest that in a variety of familial CNS disorders compromises in the 26S proteasome likely plays a more important role in mediating neurodegeneration than any potential compromise in the function of the 20S proteasome.

Numerous studies have demonstrated that proteasome inhibition occurs in PD, ALS, HD, and AD (29, 58). However, at the present time it is unclear whether the impairment in proteasome function is occurring in the 26S and/or 20S proteasome. Studies have reported that the 26S proteasome may be more vulnerable to inactivation as compared to the 20S proteasome (77). Because each of these conditions is associated with oxidative stress, these data raise the possibility that 26S proteasome function may be compromised prior to the 20S proteasome in each of these disorders. This may be particularly true in nonfamilial forms of PD, ALS, and AD which are not caused by the expression of mutant proteins. In both familial and sporadic forms of these diseases there is a pronounced increase in protein inclusion/aggregate formation. Because this observation is so common in multiple neurodegenerative disorders, it raises the possibility that the formation of an inclusion body may play a role in promoting impairments in proteasome function. It is enticing to speculate that the decades required for the onset of neuropathology and neurodegeneration in each of these conditions is due to the fact that the proteasome is able to preserve much or nearly all of its function for a considerable period of time. Once these compensatory processes are overridden, deleterious compromises in proteasome function occur, thus allowing the onset of neuropathology and neurodegeneration.

Apoptosis plays an important role during the development of the CNS, and is necessary for the formation of proper neuronal circuitry. During a variety of age-related neurodegenerative conditions there is an increase in apoptosis (7), with the apoptosis-associated neuron death contributing to the onset and progression of a host of neurodegenerative conditions. A number of the 26S proteasome substrates are involved in the apoptotic pathway (44, 98), with the best characterized of these substrates is p53. Normally a very short-lived protein, the expression of p53 is kept at a low level, and thus is unable to induce its pro-apoptotic effects. However, after inhibition of proteasome function the level of p53 would be expected to become elevated (24, 56, 70, 97), eventually elevating to the point that it is able to induce its pro-apoptotic pathways. Indeed, p53 has been demonstrated to play a causal role in the apoptosis induced by severe proteasome inhibition (70). As such, the 26S proteasome plays an important anti-apoptotic role in the CNS, preventing the aberrant activation of apoptosis. Because many apoptotic pathways involve an elevation in mitochondrial ROS generation, it is possible that the induction of apoptosis contributes to oxidative stress in the CNS of aged individuals.

Taken together, these data suggest that a fully functional proteasome (both the 20S and 26S) may play an important role as a protective barrier (Fig. 1), preventing sudden elevations in oxidative damage, preserving cellular homeostasis, and preventing the activation of apoptotic cascades. During normal aging, or as the result of stressors associated with age-related diseases of the CNS, the capacity of the proteasome to protect against stress may become compromised (Fig. 1). This compromise in function serves as a trigger, allowing the endogenous stressors to begin initiating deleterious alterations inside of the cell. In particular, compromised proteasome function would allow for the elevation in oxidative damage, induction of oxidative stress, and activation of apoptotic cascades to occur (Fig. 1).

## EVIDENCE OF PROTEASOME INHIBITION CONTRIBUTING TO OXIDATIVE DAMAGE

As described previously, mildly oxidized proteins are substrates for the 20S proteasome, with inhibition of proteasome function increasing the amount of oxidized proteins. Impairments in 20S proteasome function likely play an important role in the age-related increases in protein oxidation observed in a variety of tissues, including the CNS. It is important to note that during aging protein oxidation does not typically exhibit a gradual and progressive increase, rather during aging there is a very low level increase in protein oxidation that dramatically increases several fold in late age (54, 57, 77, 87). Proteasome inhibition may serve an important role as a trigger for the sudden and dramatic spike in protein oxidation observed in very late age. Therefore, early in the aging process there is likely a dynamic cellular environment that helps to prevent large increases in protein oxidation. For example, it is likely that proteasome plasticity and increases in stress response prevent the accumulation of oxidative damage that could potentially occur as the result of cellular stressors. Over time the ability of these protective pathways to prevent increases in protein oxidation dramatically decrease, with inhibition of proteasome function serving as a mechanism for rapidly and profoundly elevating protein oxidation. Additionally, once the levels of oxidized proteins are increased to a deleterious stage, or allowed to persist in the intracellular space for prolonged periods of time, they may serve as potent inhibitors of proteasome function. In this model, excessively oxidized proteins inhibit the entry of other proteasome substrates, thus causing inhibition of proteasome-mediated protein degradation. Con-

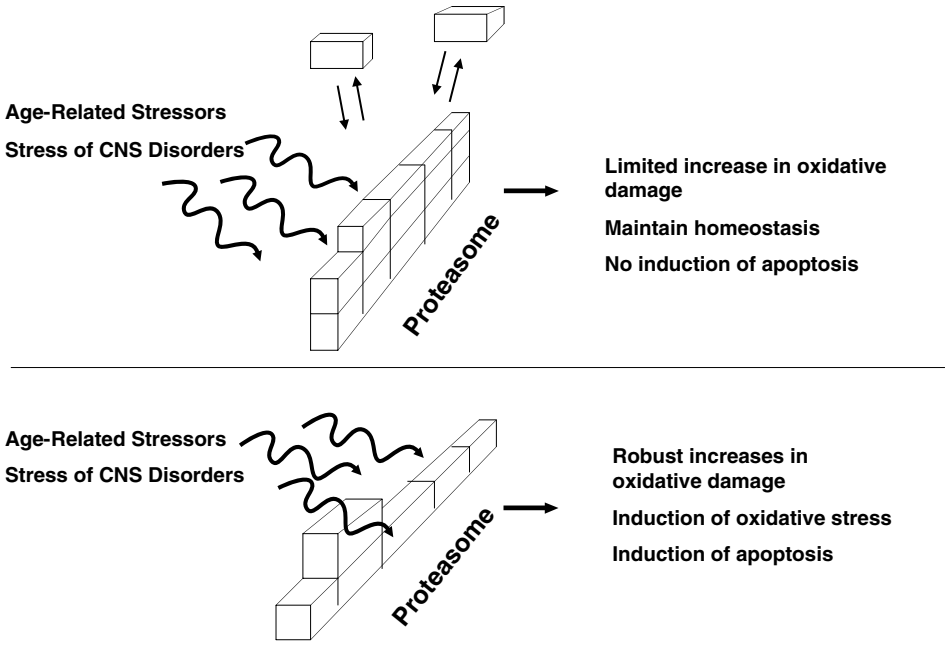


**FIG. 1. The proteasome serves as a protective barrier from stress.** The activity of the 20S and 26S proteasomes allow cells to maintain homeostasis following exposure to age-related stressor, and stressors associated with age-related diseases of the CNS. In healthy cells the amount of protection conferred by the proteasome can be rapidly increased (*top panel*) or modified in order to adequately maintain homeostasis. In contrast, aged or damaged cells (*lower panel*) there is a limited wall of protection conferred by the proteasome, and no apparent plasticity. Because of the lack of protection from the proteasome, these cells now undergo increases in oxidative stress and activation of apoptotic pathways, with proteasome inhibition serving as the trigger for initiation of these deleterious events.

sistent with this model, studies from our laboratory have demonstrated that increased heat shock protein expression ameliorates oxidative stress-induced proteasome inhibition (5, 29).

Recent studies provide direct experimental evidence for proteasome inhibition serving as a mediator of lipofuscin-ceroid (88), which is one of the most common forms of oxidative damage observed in aged tissues. Interestingly, this increase in lipofuscin-ceroid may be related to impairment in mitochondria turnover and mitochondrial function (88). Because of the importance to mitochondria dysfunction to aging and age-related diseases of the CNS, these data indicate a novel mechanism by which proteasome inhibition may contribute to neuropathogenesis. Additionally, our laboratory has demonstrated that inhibition of proteasome function (low-level inhibition) is sufficient to increase autophagy (28), which is observed in the aging CNS as well as several age-related disorders of the CNS. The chronic activation of autophagy is likely deleterious towards neural homeostasis, based on the fact that rapid and large-scale degradation of cytoplasmic complexes and organelles cannot be beneficial towards the long-term cellular viability. Therefore, induction of autophagy may serve as an additional mechanism by which proteasome inhibition contributes to cytotoxicity in the CNS. Lastly, inhibition of proteasome function in neural cells alters gene expression in a manner that is highly relevant to a variety of age-related disorders (26), including modulating the genes involved in regulating beta amyloid metabolism.

A number of studies have suggested a link between DNA repair and the proteasome. For example, the degradation of oxidized histones is mediated by the proteasome (93, 94), with additional studies showing that proteasome subunits may play a role in DNA repair (32, 62, 96). Data from our laboratory demonstrated that proteasome inhibition is sufficient to induce RNA and DNA oxidation in primary CNS cultures (27). Interestingly, nucleic acid oxidation occurred in neurons and astrocytes, although it was much more severe in neurons as compared to astrocyte cultures. The oxidation of RNA was associated with an alteration in RNA processing. These data suggest that there is potential crosstalk between proteasome-mediated protein degradation and the translation/protein synthesis processes. The proteasome is also capable of increasing ROS production (25, 35, 65, 88), which can increase oxidative stress. Studies have shown that both severe and moderate proteasome inhibition are capable of stimulating ROS generation in neural and non-neural cells. In at least one study, the increase in mitochondrial derived ROS has been reported (88).

Together, these data that there are multiple mechanisms by which proteasome inhibition can contribute to increased oxidative damage, and potentially the induction of oxidative stress. The ability of proteasome inhibition to induce so many disparaging effects should not be considered surprising when one considers the large number of proteasome substrates, and the likelihood that alterations in bulk protein turnover may impact multiple systems. The fact that proteasome inhibition is capable of inducing so many forms of age-related oxidative damage, and the fact that proteasome inhibition appears to be a common occurrence in aging, these data suggest that proteasome inhibition has to be considered seriously as a mecha-

nism for increasing oxidative damage during normal aging. Therefore the proteasome should not only be thought of as a target of ROS, but also be discussed in the context of proteasome inhibition being a potential mediator of oxidative stress. We propose that in healthy cells the activity of the proteasome is compromised for a small period of time (Fig. 2), but the presence of antioxidants, heat shock proteins, and proteasome plasticity are sufficient to re-establish proteasome function. The fact that proteasome inhibition is only allowed to persist for a short period, the induction of oxidative stress, and activation of apoptotic pathways can be avoided (Fig. 2). In older cells it is likely that following cell stress proteasome inhibition is sustained due to the fact that antioxidant pathways, heat shock protein response, and proteasome plasticity are insufficient (Fig. 2). This sustained inhibition of proteasome function allows for elevations in oxidized macromolecules, activation of apoptotic pathways, and increased levels of protein aggregation to occur (Fig. 2). Ultimately, the cell stressors induced by proteasome inhibition promote a feed-forward pathway that compounds the amount of proteasome inhibition and induction of pathology that is observed (Fig. 2).

## ROLE OF PROTEASOME INHIBITION IN AGING AND AGE-RELATED DISORDERS OF THE CNS

Proteasome inhibition appears to be significantly correlated with the onset of cellular senescence (5, 10, 13, 38, 84), observed in multiple cell types during the onset of cellular senescence *in vitro*. These data were suggested to indicate a role for proteasome inhibition as a mediator of senescence. Studies with pharmacological inhibitors have strengthened this concept, demonstrating that proteasome inhibition is sufficient to induce senescence and multiple features of senescence (12). Thus it appears that proteasome inhibition may play a direct role in mediating cellular senescence, although the molecular basis for such involvement remains to be elucidated. In future studies it will be important to expand these studies and elucidate the exact contribution maintenance of proteasome function may play in regulating lifespan.

Proteasome inhibition has been reported to occur in a number of neurodegenerative conditions including ALS, PD, HD, and AD. It is important to note that the inhibition of proteasome function in these conditions is fully distinguishable from the normal age-related impairments in proteasome function. It will be important to elucidate in future studies if this exacerbation represents the presence of alternative mechanisms for proteasome inhibition, or whether these data represent an increased activation of pathways which are capable of inducing proteasome inhibition. For example, proteasome inhibition in ALS, PD, and HD may result from increases in protein aggregations that are not observed in normal aging. Some studies reported finding proteasome subunits in a variety of protein inclusions (11, 18), which was suggested to indicate that proteasome inhibition was being mediated by a sequestration of proteasome complexes into protein aggregates. It is important to point out that the presences of proteasome subunits (which can account for 1–2% of total cellular protein) do not indicate any evidence for proteasome complexes being

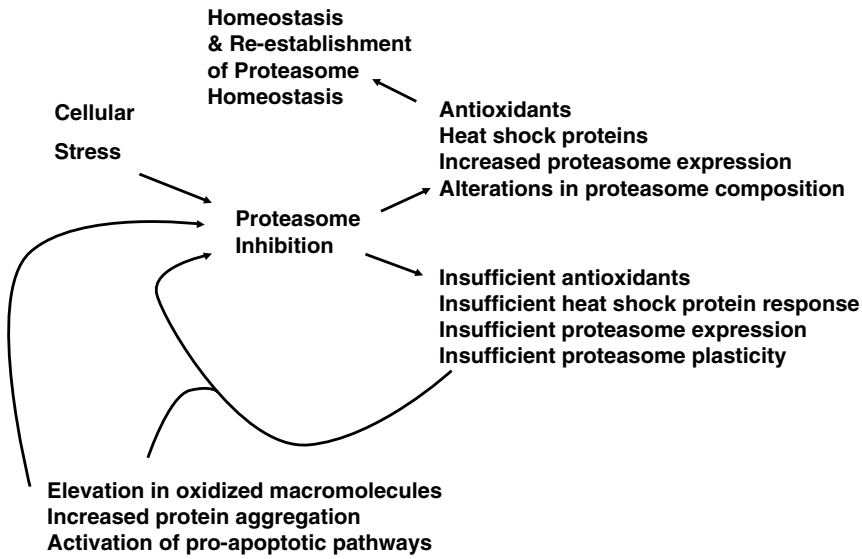


**FIG. 2. The proteasome plays dual roles in the onset of oxidative stress.** In healthy cells the function of the 20S and 26S proteasome is inhibited briefly following exposure to cell stress. Proteasome function is only inhibited briefly, due to the presence of antioxidants, heat shock proteins, and proteasome plasticity. As such, cells resume normal proteasome function and return to homeostasis. In contrast, in unhealthy cells there is a sustained low-level inhibition of the proteasome following exposure to a cell stressor. This inhibition is allowed to persist and allows for the induction of oxidative stress and the activation of apoptotic pathways. Increases in oxidative stress and apoptotic pathways cause a feed-forward cycle that results in more severe proteasome inhibition and greater amounts of oxidative stress and activation of apoptotic pathways. In this model, proteasome inhibition is the trigger by which increases in oxidative stress and apoptotic pathways are increased in the CNS.

present in protein inclusions. In fact it would be shocking if these highly expressed proteasome subunits were not present in proteinaceous inclusions due to the pervasiveness of their expression, and it remains to be elucidated if proteasome complexes are ever sequestered into protein aggregates.

A number of studies have demonstrated that inhibition of proteasome function is sufficient to induce the activation of caspases, mitochondrial dysfunction, protein aggregation, and apoptosis (24, 31, 63, 76, 78). These *in vitro* studies were taken to suggest that proteasome inhibition is sufficient to induce neurotoxicology relevant to a variety of neurodegenerative conditions. A recent *in vivo* study demonstrated that *in vivo* administration of proteasome inhibitor was sufficient to induce neuropathology and motor deficits relevant to PD in rodents (68). These data demonstrate that proteasome inhibition is sufficient to induce neuropathology and neurophysiological abnormalities relevant to PD *in vivo*. At the present time data are still needed to elucidate whether proteasome inhibition is necessary to induce neuropathology and neurodegeneration in age-related disorders of the CNS.

So if proteasome inhibition is a potentially important mediator of pathogenesis, how can proteasome inhibition be preserved during normal aging or in age-related disease? Our best hints of such regulation come from studies involving caloric restriction (CR). In studies of CR an amelioration of age-related proteasome inhibition was reported (69, 89). Interestingly, the preservation of proteasome function was associated with reduced levels of protein oxidation. These data raise the possibility that preservation of proteasome function may serve as a mechanism for ameliorating age-related increases in oxidative damage. A number of CR mimetics have been designed, and may be able to positively impact proteasome function during normal aging. Additionally, compounds which preserve or enhance HSP response may be beneficial in preserving proteasome function during normal aging, by ensuring the targeting and unfolding of proteasome substrates. Lastly, genetic transfer of endogenous proteasome activators may serve as useful mechanisms for elevating proteasome function in targeted cell populations.

## ABBREVIATIONS

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; HNE, 4-hydroxy-2-alkenal; PD, Parkinson's disease.

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