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Forum Review

Proteasomal Dysfunction: A Common Feature of Neurodegenerative Diseases? Implications for the Environmental Origins of Neurodegeneration

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ABSTRACT

The neurodegenerative diseases that afflict humans affect different part of the nervous system and have different symptoms and prognoses, yet they have certain things in common. One of them is defects in the clearance of abnormal or other "unwanted" proteins, particularly affecting the proteasome system. In this review, I advance two concepts: (a) that defects in protein clearance can be a fundamental cause of neurodegeneration, and (b) that because proteasome inhibitors are widespread in nature, their ingestion may contribute to "spontaneous" neurodegeneration. *Antioxid. Redox Signal.* 8, 2007–2019.

INTRODUCTION: SETTING THE SCENE

Neurodegenerative diseases have different symptoms, affect different parts of the nervous system, and may often have different causes (21, 62, 64, 66, 111, 147, 156). However, contributions to understanding their pathogenesis may come from realizing that they have several features is common: impaired mitochondrial function, increased oxidative damage, the presence of abnormal, aggregated proteins, changes in iron metabolism (Table 1), and some involvement of excitotoxicity and of inflammation. The proteins may be "abnormal" because they are the product of mutant genes (as in FALS and the inherited variants of PD and AD), but in most cases, the amino acid sequences are "correct," but the proteins have been modified chemically (Table 2). Increased oxidative damage in the neurodegenerative diseases is manifested as increases in lipid peroxidation end products (*e.g.,* F_2-isoprostanes, 4-hydroxynonenal, acrolein, and sometimes F_4-isoprostanes), DNA (and often RNA) base oxidation (usually measured as 8OHdG or 8OHG), and protein damage. The protein aggregates frequently contain proteins that are nitrated, bear carbonyl residues, have attached aldehydes such as HNE or acrolein, and, sometimes, carry AGE products (6, 15, 21, 41, 56, 62, 64, 66, 90, 110–112, 125, 147, 156, 169).

THE ROLE OF THE PROTEASOME

In nondividing cells, such as most neurons in the adult brain, the protein content is approximately constant. Because protein synthesis is continuous, it must be matched by an equal rate of protein degradation. Cellular proteins can be degraded by the lysosomal system, whose importance to the brain is clearly revealed by the pathology of the neuronal ceroid lipofuscinoses (109). However, a system of equal or greater importance to the normal functioning of the nervous system is the proteasome. The ubiquitin–proteasome system is essential to the development and maintenance of neurons (29, 141), and also plays a role in axonal degeneration after nerve injury (91).

I first became interested in this system in 1998 (67), when we realized that the accumulation of oxidized and other abnormal proteins observed by us (2, 64, 101, 102) and others (22, 27, 41, 47, 56, 115, 136, 147) in neurodegenerative diseases could be due not only to increased oxidative damage but also to failure to clear damaged proteins (36, 61, 67, 141). In mammalian cells, oxidized proteins appear to be largely removed by the 20S proteasome (35, 38, 60, 149, 155), an exception being removal of oxidized aconitase by the mitochondrial Lon protease (16). Surprisingly, perhaps, ubiquitination

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TABLE 1. THE NEURODEGENERATIVE DISEASES–WHAT THEY HAVE IN COMMON

	Parkinson disease	*Alzheimer's disease*	*Amyotrophic lateral sclerosis*	*Freidreich's ataxia*	*Huntington's disease*	*Prion diseases*
Mitochondrial dysfunction	Complex I ↓, αKGDH ↓	Complex IV ↓ (some studies), αKGDH* ↓, Pyruvate dehydrogenase ↓	Complex I and IV ↓ (varying reports). Mitochondrial dysfunction very obvious in transgenic mouse models expressing mutant SODs related to FALS.	Frataxin is a mitochondrial Fe–S protein; levels of complexes I, II, III and aconitase decreased.	Complexes II, III ↓, αKGDH ↓, aconitase ↓, Mutant huntingtin may bind to and damage mitochondria.	Defects reported in brains of scrapie–infected mice.
Proteasome dysfunction	Specific genetic defects in this pathway cause inherited PD (Fig. 2). Proteasome proteolytic activities sub-normal in sporadic PD.	Proteasome proteolytic activities sub-normal. One reason may be the presence of UBB^{+1}, a mutant ubiquitin carrying a 19-amino acid C-terminal extension that is found in affected neurons in AD and Down's syndrome, apparently generated by errors during transcription. This mutant ubiquitin cannot attach itself to an expanding polyubiquitin chain, and also appears to inhibit the proteasome.	Proteasome activity may be decreased by aggregates of mutant SODs in FALS (26).	No data as yet.	Protein aggregates include proteasome subunits and may impair proteasome function.	Accumulation of ubiquitinated proteins observed in animal models and CJD brain suggestive of proteasome dysfunction (6).

TABLE 1. CONTINUED

	Parkinson disease	*Alzheimer's disease*	*Amyotrophic lateral sclerosis*	*Freidreich's ataxia*	*Huntington's disease*	*Prion diseases*
Abnormal protein aggregates	Lewy body	Amyloid plaques, diffuse amyloid deposits, neurofibillary tangles.	A range of aggregates described, often containing ubiquitin, neurofilaments, dorfin† etc in motor cortex and spinal motor neurons. CuZnSOD is a major component of aggregates in FALS caused by SOD1 mutations.	Frataxin aggregates in nucleus.	Aggregates containing huntingtin, ubiquitin, heat-shock proteins and proteasome subunits.	Abnormal protein aggregates. Fragments of PrP^c are toxic to neurons in culture.
Changes in iron metabolism	More iron in substantia nigra.	Iron in plaques.	Iron deposition in dying motor neurons.	"Catalytic" Fe levels might be elevated due to abnormal frataxin, although this has not been shown experimentally (132, 143). Iron deposits in heart.	Iron deposited in lesions.	Iron levels raised in affected areas (90).
Oxidative and nitrative damage*	Revealed by multiple biomarkers of oxidative damage.	Revealed by multiple biomarkers of oxidative damage.	Revealed by multiple biomarkers of oxidative damage.	Revealed by multiple biomarkers of oxidative damage.	Some evidence for elevated levels of 8OHdG and lipid peroxidation.	Brains show increased lipid peroxidation and oxidative protein damage.

†Dorfin is an E3 ubiquitin ligase (113), also found in Lewy bodies.

αKGDH, α–ketoglutarate dehydrogenase; FALS, familial amyotrophic lateral sclerosis; CJD, Creutzfeldt–Jakob disease; 8OHdG, 8-hydroxy-2′-deoxyguanosine; AD, Alzheimer's disease; PD, Parkinson disease. Adapted from ref. 66 by courtesy of Oxford University Press. *Please see refs. 62, 64, 66, and 84a for detailed references except where quoted in the Table.

Table 2. Processes Generating Abnormal Proteins in Neurodegenerative Diseases

Overexpression of a normal gene, causing too much normal protein to accumulate (*e.g.,* triplication of the synuclein gene in some rare familial cases of PD)
Gene mutations, producing an abnormal protein
Aberrant splicing of mRNA, producing an abnormal protein
Faulty post-translational modification, producing an abnormal protein
Oxidation of amino acid residues by reactive oxygen species
Nitration and/or oxidation of amino acid residues by reactive nitrogen species
Halogenation and/or oxidation of amino acid residues by reactive chlorine or bromine species*
Glycation/glycoxidation†
Spontaneous deamidation or deamination
Modification by end-products of lipid peroxidation such as HNE, other aldehydes and isoketals
Modification by end-products of the cyclooxygenase pathway, *e.g.,* cyclopentenone prostaglandins, levuglandins
Modification by quinones/semiquinones, arising from oxidation of L-DOPA, dopamine, serotonin, noradrenalin and other autoxidizable biomolecules in the brain

Adapted from ref. 66 by courtesy of Oxford University Press.

*The enzyme myeloperoxidase, which uses H_2O_2 to oxidize Cl^- to HOCl, is not normally present in brain but has been reported to appear in both AD and PD brain, and can lead to protein chlorination (56).

†Glycoxidation involves both glycation and oxidation of proteins, forming advanced glycation end (AGE) products that impair protein function and can be cytotoxic (66).

does not appear to be required for degradation of oxidized proteins (35, 60, 134), except in a few special cases. One of these is iron-regulatory protein 2 (IRP2), which plays a role in regulation of cellular iron metabolism and is especially important in the brain; knockout of the gene in mice causes iron deposition and neuronal damage (55). Oxidized IRP2 is recognized by an E3 ubiquitin–protein ligase (166). So how does the 20S proteasome recognize other oxidized proteins? The answer is not clear; one suggestion is that oxidation increases surface hydrophobicity, but more studies to investigate the mechanisms by which this could trigger recognition are needed (35, 60, 155). Heat-shock proteins may also be involved (161).

Levels of oxidized proteins in brain, as measured by "global" biomarkers such as protein carbonyls (31, 68), tend to increase with age (87, 136), consistent with reports that proteasome activity decreases with age (23, 87, 88, 141, 149). Lon protease activity also decreases with age (9). Some animal studies suggest that levels of brain protein carbonyls are positively correlated with the degree of cognitive impairment (22, 47, 136). Further evidence of a link between these two phenomena is provided by an observation (in gerbils) that the spin trap *tert*-butyl-α-phenylnitrone (PBN) both decreased carbonyl levels and improved cognitive function (22). Caloric restriction also decreased brain protein carbonyls and improved cognitive abilities in mice (40). A relation of oxidative protein damage to neuronal dysfunction is very likely because the oxidized proteins include enzymes essential to neuronal energy metabolism, such as triose phosphate isomerase and α-enolase (147) and components of complex I (84a).

Increased levels of nitrated proteins have been observed in nervous tissues from subjects with AD, PD, HD, or ALS (17, 41, 57, 69, 144), although some of the earlier studies may need reevaluation because of methodologic artifacts (49, 83, 130, 152). Thus, simple HPLC determinations of 3-nitrotyrosine in brain tissue can be confounded by co-eluting peaks (83), and exposure of tissues or body fluids to acid (*e.g.*, to hydrolyze proteins to release 3-nitrotyrosine) can cause artefactual nitration (49, 130, 152). The increase in nitrated protein levels is usually assumed to be caused by generation of more reactive nitrogen species (RNS), such as peroxynitrite $ONOO^-$ (41). However, nitration is not a specific marker of damage by $ONOO^-$; it can be caused by several reactive species, RNS (65). For example, myeloperoxidase is present in the brain in some neurodegenerative diseases (56), and this enzyme can catalyze protein nitration (10, 65). It can also oxidize Cl^- ions to hypochlorous acid (HOCl), which can chlorinate proteins (56).

An increase in levels of nitrated products could indeed be due to greater RNS generation, but it could also be due to (at least in part) to failure to remove them at normal rates. How nitrated proteins are cleared *in vivo* is uncertain; "denitrase" enzymes (of unknown structure) have been described (78), but proteasomal degradation of nitrated proteins may also be important (59, 138). How the proteasome might recognize them is unknown. The fate of chlorinated proteins is even less clear. Degradation of nitrated proteins would presumably release free nitrotyrosine. However, reports of elevated free nitrotyrosine levels in CSF from ALS patients have not been confirmed (130), and it may be in any case that free nitrotyrosine is rapidly degraded. The role of ubiquitination in degrading nitrated proteins is uncertain; in bovine aortic endothelial cells, degradation of nitrated transferrin receptor did involve ubiquitination (93), although isolated 20S proteasome without the ubiquitin system is able to degrade nitrated CuZnSOD (138).

THE PROTEASOME AND OXIDATIVE STRESS

My interest in the proteasome deepened when we examined the effect of adding proteasome inhibitors to cells in culture, initially NT-2 (a human teratocarcinoma) and SK-N-MC (a human neuroblastoma) cell lines. Addition of the proteasome inhibitors lactacystin or epoxomicin to either cell type caused apoptotic death. Apoptosis was delayed (but not prevented) by adding NOS inhibitors, and accelerated by adding more L-arginine to the cell culture medium. Production of extra $NO^{\bullet}$ was demonstrated, because of an increase in nNOS levels (97), presumably because this protein is normally degraded by the proteasome (120). There was also an increase in levels of nitrated proteins, protein carbonyls, and other markers of oxidative damage and a decrease in mitochondrial metabolic activity, as revealed by a rapid decline in the ability to reduce MTT (97). Thus, inhibiting the proteasome leads to oxidative stress (97, 141). Similarly, proteasome inhibition

led to increased DNA and RNA oxidation in primary neurons (36). In liver cells, formation of protein aggregates and cell death induced by adding lactacystin were decreased by lowering O_2 levels, further consistent with a role of reactive species in damage induced by proteasome inhibition (34). Oxidative stress also contributes to cell death induced by proteasome inhibitors in leukemia (32) and lymphoma (121) cells. Exactly why proteasomal inhibition causes oxidative stress is uncertain. In NT-2 and SK-N-MC cells, oxidative stress may increase intracellular Ca^{2+} levels (66), which would activate the accumulated nNOS (because it is Ca^{2+}-calmodulin dependent) and thus produce more NO•. This can react with $O_2^{\bullet -}$ to form $ONOO^-$, promoting protein nitration. Similar results were reported in SH-SY5Y neuroblastoma cells overexpressing an ALS-related mutant CuZnSOD; the cell death induced by adding lactacystin could be ameliorated by the nNOS inhibitor 7-nitroindazole (7). The toxic effects of proteasome inhibitors are aggravated if cells are overexpressing abnormal proteins, such as mutant CuZnSOD, α-synuclein, or parkin (14, 74, 76, 141). Interestingly, interference with the ubiquitination process by using a dominant-negative form of ubiquitin also caused increased NO• production and protein nitration in NT-2 and SK-N-MC cells, as well as decreased proteasome activity (73). In other words, interference with the ubiquitin–proteasome system at any point may be able to cause oxidative and nitrative stress, impair cell function, and increase sensitivity to neurotoxins such as HNE, H_2O_2, mitochondrial complex I inhibitors, and neurotoxic metal ions such as Cd^{2+} (73, 74, 76, 141). Other sources of reactive species in cells with inhibited proteasome activity include increased mitochondrial ROS production (98, 133, 146) and more generation of $O_2^{\bullet -}$ by the activation of NADPH oxidase enzyme complexes (164).

Many different effects of proteasome inhibitors are described in the literature, with a wide range of cells. They include causing neurite outgrowth [that was how the widely used inhibitor lactacystin was discovered (43)] and *protection* of cells against apoptosis, *e.g.*, by preventing activation of NF-κB (18,140), by modulating mitochondrial function (145), or by raising levels of heat-shock proteins (39, 124, 167). Activation of NF-κB can be prevented because iκB is degraded through the proteasome (18). Cyclins involved in regulation of cell division are degraded by the proteasome, and so its inhibition dysregulates the cell cycle (43, 118). Inhibition of the proteasome can also raise cellular p53 levels, again promoting shut-down of cell division. This is because the E3 ubiquitin ligase MDM2 targets p53 for degradation by the 26S proteasome (157).

These variations in published results may be due to the use of different cell types (*e.g.*, dividing vs. nondividing), inhibitors of different types applied at different concentrations achieving various degrees of proteasomal inhibition, different modes of cell death (100), or different observation periods (see later). Nevertheless, it is clear that a sufficient degree of proteasomal inhibition causes apoptosis (or cell death by other mechanisms) in neurons (153) or relevant cell lines. This has been shown in cerebellar granule cells (123), neonatal mouse sympathetic neurons (94), mouse cortical neurons (148, 167), NT-2 (97), SK-N-MC (97), and PC12 cells [both naive and neuronally differentiated (128)], and in rat oligodendrocytes (52). Application of lactacystin to slices of rat neonatal spinal cord produced death of motor neurons (153). Proteasome inhibition also caused cell death and activation of poly(ADP-ribose) polymerase (PARP-1) in the PC6 cell line; inhibition of this enzyme decreased cell death (85). Although high levels of proteasome inhibitors are cytotoxic to primary mouse cortical neurons, low levels tended to prolong cell viability in culture, associated with increased levels of heat-shock proteins (141, 167) and a range of changes in gene expression, including upregulation of the expression of genes encoding proteasome subunits (167). The increase in Hsps may be triggered by the early stages of accumulation of abnormal proteins and will maintain survival only if their chaperone activities can cope with the amounts of abnormal protein present (Fig. 1). It is important in such studies to follow the cells for as long a period as possible; what appears initially to be neuroprotection might switch to accelerated cell death as proteins continue to pile up. In some cases, Hsps might even facilitate protein aggregation (165).

Proteasome inhibition in NT-2 and SK-N-MC cells provoked the formation of protein aggregates in the cell cytoplasm; among the constituents were α-tubulin, ubiquitin, CuZnSOD, α-synuclein, and 68K neurofilaments (75). Nitrotyrosine was also present, and aggregate formation was decreased by NOS inhibitors, consistent with suggestions that nitration may facilitate aggregate formation (41, 75, 119). Similarly, introducing a mutant proteasome subunit that decreased the chymotrypsin-like activity hypersensitized mouse neuronal cells to oxidative stress, and such stress resulted in protein aggregate formation (99). Of course, NO• is probably not the only mediator of cell death induced by proteasome inhibition; activations of PARP-1 (85) and of COX-2 [with subsequent increased prostaglandin production (129)] may also be important, depending on the cell type used and its growth conditions.

Intense debate occurs about whether inclusion bodies or other protein aggregates are toxic to neurons. In general, it may be the early stages of aggregate formation (*e.g.,* of huntingtin or β-amyloid) that are toxic rather than the final insoluble complexes; formation of the latter may be beneficial if it helps convert toxic oligomers to an insoluble form (115). Some cells handle aggregated proteins by moving them all to a single site in the cell, the aggresome, which can then be dealt with by uptake into lysosomes. This mechanism seems to fail in the neurodegenerative diseases (160). But how could partially aggregated proteins be toxic? We examine this question next.

IS THE PROTEASOME DYSFUNCTIONAL IN NEURODEGENERATIVE DISEASES?

Yes, it is. First, several inherited forms of PD involve defects in the ubiquitin–proteasome system (Fig. 2). However, what about the much more common sporadic forms? Yes, again (105, 106, 141). Levels of proteasome activity are also decreased in AD (86), after cerebral ischemia–reperfusion (8, 163) or intermittent hypoxia (54), in prion diseases (71), and possibly in some cases of schizophrenia (4). In transgenic

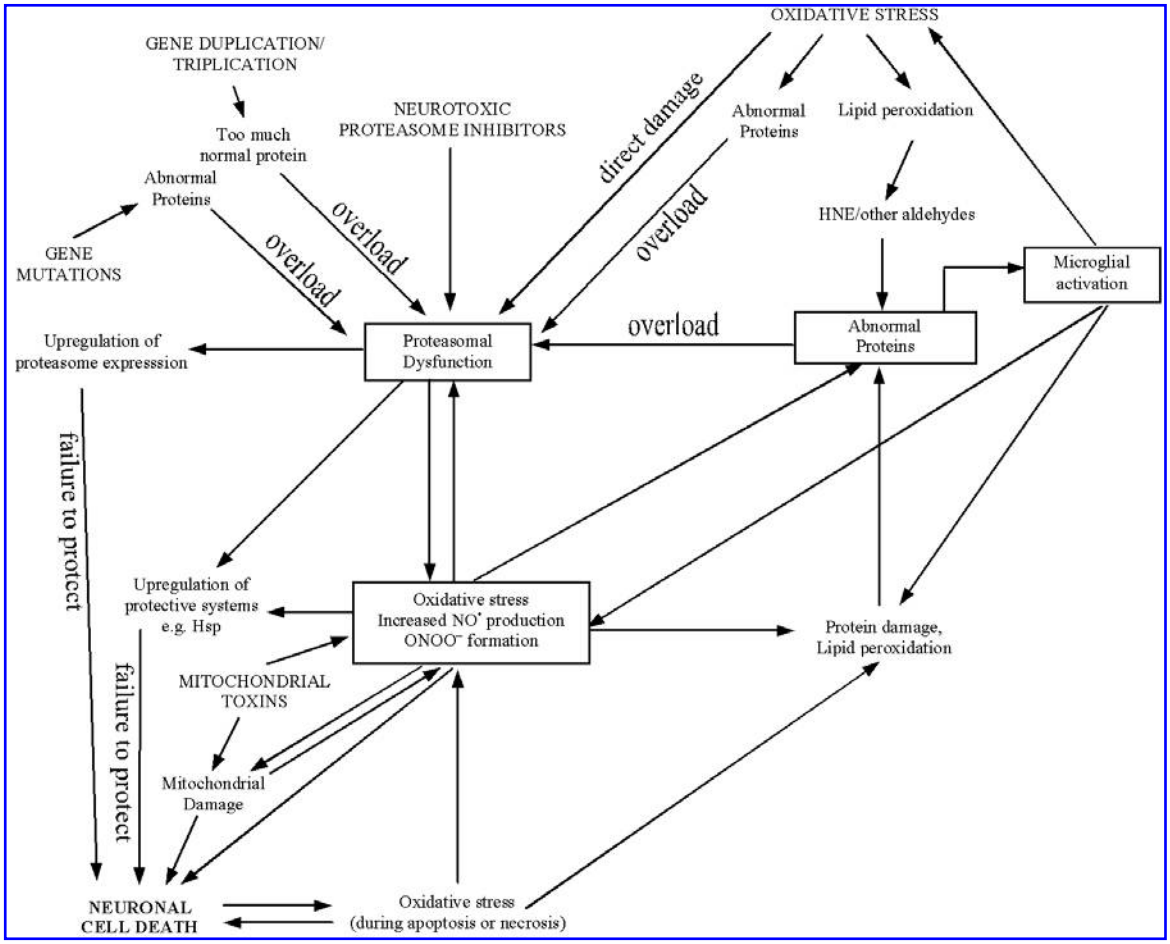

FIG. 1. Interplay of mitochondria, oxidative damage, and the proteasome in neurodegeneration. Low-level proteasome inhibition can cause transient neuroprotection [*e.g.,* by induction of heat-shock proteins (Hsps)] (39, 141, 167). Adapted from ref. 66 by courtesy of Oxford University Press.

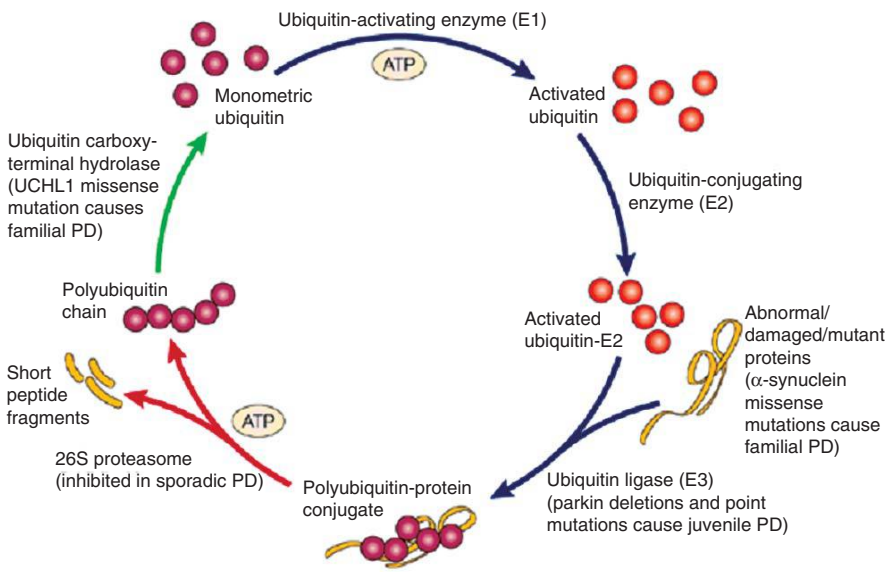

FIG. 2. Defects in the ubiquitin–proteasome system in Parkinson disease (PD). Blue section shows the normal ATP-dependent identification and labeling of unwanted proteins with ubiquitin molecules (ubiquitination) as a signal for ATP-dependent degradation by the 26S proteasome complex (proteolysis; red section). Green section shows recovery and recycling of ubiquitin molecules that are released from proteins. Also depicted are ways in which potential defects in the system cause PD. UCHL1, one of the most abundant proteins in brain (≤2% of total brain protein) releases free ubiquitin and allows the cycle to continue. A variety of deletion and point mutations in the parkin gene can lead to PD, and mice lacking parkin show neuronal cell death in various parts of the brain (159). From ref. 106 by courtesy of Prof. Peter Jenner and *Nature* publishers.

mice expressing ALS-associated mutant CuZnSOD, proteasome activity in the spinal cord was decreased (82).

How could such decreases occur?

Overload and Inhibition

Abnormal proteins arise by a variety of mechanisms (Table 2), and many are degraded by the proteasome system. The presence of such proteins may "overload" the system, either if they are degraded more slowly than usual and "clog up" the system (11, 64) or if the cell "senses" that they are abnormal and turns them over faster, in either case requiring a greater total amount of "proteasomal time." For example, the mutant α-synucleins associated with some cases of familial PD appear to be degraded more slowly than normal α-synucleins (12, 141). Expressing them in PC12 or neuroblastoma cells led to decreased proteasomal activity (128, 151), as did cellular expression of some of the mutant CuZnSOD enzymes associated with ALS (3, 75, 77). Parkin is also degraded by the proteasome, the mutant parkins associated with juvenile PD apparently abnormally slowly (28, 76). The LI66P mutant DJ-1 protein associated with a few familial PD cases is also degraded by the proteasome (108). Paired helical filament tau has been reported to block proteasome function and may contribute to decreased proteasome activity in AD (84); another possible "blocker" is Alzheimer-associated variant ubiquitin (72), UBB^{+1}.

Abnormal proteins need not always interfere directly with the proteolytic activities of the proteasome. Another scenario is that abnormal proteins bind to the cap structures and impair the binding of ubiquitinated proteins, or their feeding into the proteasome core (137). In SH-SY5Y cells, expanded polyglutamine repeat proteins did not markedly decrease proteasome function, but they did significantly impair the cells' ability to increase proteasome levels in response to thermal stress, illustrating yet another potential mechanism (37).

Overload of the proteasome can also be caused by the presence of increased levels of normal proteins [*e.g.*, of α-synuclein (in the rare inherited cases of PD caused by triplication of the α-synuclein gene) or CuZnSOD (in Down syndrome)]. Excess generation of oxidized, nitrated, and possibly chlorinated proteins could have the same effect. For example, isoketal-modified, HNE-modified (including HNE-modified β-amyloid), and possibly acrolein-modified proteins, can decrease proteasome function by attempting to enter and getting "stuck," being unable to be rapidly degraded (48, 58, 133, 134). For example, in SK-N-MC or NT-2 cells treated with HNE, this cytotoxic aldehyde became associated with the proteasome (77), either by direct binding of it to proteasomal subunits and/or by association of other HNE-modified proteins with the proteasome.

Damage to the proteasome

Several authors have suggested that reactive species can attack the proteasome and directly inactivate its hydrolytic activities (141). Glockzin *et al.* (51) suggested that $NO^{\bullet}$-induced apoptosis in RAW264.7 macrophages involves proteasomal inhibition, although $NO^{\bullet}$ is probably itself insufficiently reactive to attack the proteasomal proteinases (66). How sensitive is the proteasome as a direct target of oxidative damage? High levels of HOCl and $ONOO^{-}$ rapidly inactivate the protease activities of the isolated proteasome (5, 30, 127, 141) under certain conditions, as can hydroxyl radical (142). Some studies have reported activation of proteinase activity on exposure to reactive species, or both activation and deactivation, depending on the concentration of the reactive species used (5, 116, 142, 158). One point worth making is that many scientists use small fluorogenic substrates to measure proteasome function; their hydrolysis is independent of ubiquitination (*i.e.*, they would not detect impairments of the ubiquitin–proteasome system at the preproteasome level), and, more relevant to this section, the proteinase levels measured can be affected by the presence of agents that can activate latent hydrolytic activities (*e.g.,* by "opening up" the proteasome). The "activations" by reactive species may be due to damage to the proteasome structure, exposing the catalytic sites; which would not be good for the cell! We found (unpublished) that low levels ($\leq$100 μM) of HNE had no effect on isolated proteasomes. Similarly treatment of NT2 or SK-N-MC cell lines with HOCl led to no significant decrease in proteasome activity even at 3 h, and indeed a slight (but nonsignificant) trend to an increase (77). By contrast, HNE did decrease proteasome activity in neural PC6 cells (87) and a motor neuron cell line (88). Lipid peroxides (158), aldehydes such as glyoxal (19), dopamine oxidation products (89), and isoketals (33), reactive products generated by the pathway that leads to isoprostane formation during lipid peroxidation, are also potential inhibitors. These actions could be relevant because levels of dopamine oxidation products (81, 139) and products of the isoprostane pathway (44) are elevated in PD. Levels of products originating from the isoprostane pathway are elevated in most or all neurodegenerative diseases (13, 44, 62, 110). In addition, quinone and semiquinones generated by oxidation of L-Dopa or dopamine can bind to proteins, facilitating aggregation and overloading the proteasome (139, 168).

In general, however, the 20S proteasome appears less sensitive to oxidative damage than the 26S proteasome (19, 79, 126, 127), suggesting that the regulatory complexes may be more important targets. Another complicating factor is that oxidative stress, if not too intense, can upregulate the expression of genes encoding proteasome subunits and increase proteasome levels (39, 53, 141, 154, 167). My overall impression from the literature is that the 20S proteasome is not very sensitive to direct inactivation by reactive species. However, the importance of its rapid inactivation by isoketals (33) needs more study. More information is also needed about the sensitivity to, and mechanism of, oxidative damage to the cap structures in the 26S proteasome.

In addition, reactive species might affect other steps in the ubiquitin–proteasome system. It has been proposed that E1 and E2 enzymes are reversibly inhibited by oxidized glutathione (*i.e.*, their activities could be impaired by oxidative stress–dependent decreases in cellular GSH/GSSG ratios) (80, 114). This occurs because GSSG can react with protein −SH groups essential to catalytic function, converting them into mixed disulfides in a process often called *S*-glutathionylation (66). More work to explore the physiologic significance of this is required. It could be particularly relevant to PD, in which GSH levels in the substantia nigra are signifi-

cantly depleted, accompanied by increases in the levels of cysteinyl–dopamine conjugates, indicative of dopamine oxidation (81, 139).

A VICIOUS CYCLE?

Neurodegeneration could start with impaired proteasome function (*e.g.*, due to chemical inhibition, age-related decline in activity, or genetically determined low levels of proteasome function), allowing abnormal proteins to accumulate and aggregate, and causing oxidative stress (Fig. 2). It could start with oxidative stress, causing neuronal damage, possible direct proteasomal damage, and proteasome overload with oxidized and/or nitrated and/or chlorinated proteins. Finally, neurodegeneration could start with defects in mitochondria (62, 169). Reactive species generated in mitochondria (*e.g.*, due to defects in the electron transport chain) can affect the proteasome, sometimes (at least in part) by decreasing ATP levels (70). In neuroblastoma cells treated with rotenone, a decrease in proteasome activity was associated with its modification by acrolein, suggesting also the possibility of direct damage (133). By contrast, loss of viability in primary rat neurons induced by another complex I inhibitor, MPP^+, was accompanied by *increased* proteasome activity (131), an attempt at adaptation, perhaps? Hence one should be cautious about generalizing to the *in vivo* situation from experiments on a single cell line, especially as cell-culture conditions can have profound effects on cell behavior (63), including proteasome activities (50). However, long-term infusion of MPTP into mouse brain led to proteasome inhibition (46). Yet another clue pointing to a key role for mitochondria is provided by the observation that early-onset PD can be caused by mutations in the nuclear gene encoding a mitochondrial protein, PINK1, a protein kinase that is somehow able to protect cells against apoptosis induced by proteasome inhibition (111).

Let us further consider PD as an example of how neurodegeneration could be triggered by mitochondrial damage, proteasome dysfunction, or oxidative stress. In some studies, treatment of rats or monkeys with low doses of the complex I inhibitor rotenone over long periods produces PD-like symptoms and neurodegeneration accompanied by oxidative damage, nitrotyrosine formation, and generation of inclusion bodies containing α-synuclein (125). Unlike MPP^+, rotenone does not concentrate in dopamine neurons, yet it can still induce fairly selective neurodegeneration in the substantia nigra (SN). It follows that SN neurons may be especially sensitive to complex I inhibition, so that any toxin affecting complex I might cause PD-like neurodegeneration (20, 25, 62). Such toxins may be widespread in the environment; even rotenone in some places (20, 25, 162). So might proteasome inhibitors; many are natural products (43). The phenolic "antioxidants" BO-653 and probucol were reported to decrease the gene expression and levels of the proteasome in human endothelial cells (150), suggesting that many more agents than we currently suspect may modulate proteasome function.

However, PD need not always start with mitochondrial defects. Studies with 6-hydroxydopamine show that oxidative stress can cause neurodegeneration (125). Dopamine oxidation products (which accumulate in PD) can both damage mitochondria and inactivate the proteasome. The effects of mutations in the ubiquitin–proteasome system (Fig. 2), together with the finding that UCHL1 activities are decreased even in sporadic PD (27), suggest that all the events shown in Fig. 1 are important. This decrease in UCHL1 activity involves oxidative damage, because the protein shows elevated levels of carbonyls and methionine sulfoxide (27). Mice lacking UCHL1 show widespread neurodegeneration, formation of protein aggregates, and increased oxidative damage (24). Mice with defective parkin show mitochondrial dysfunction and oxidative damage (117). Further evidence for close linkages between all these phenomena comes from studies with the DJ-1 protein—several mutations in the gene encoding this cause autosomal recessive PD. It has been speculated that DJ-1 has several functions, including acting as an antioxidant (it has an easily oxidizable −SH residue) that translocates to mitochondria under conditions of oxidative stress (111). Abnormal DJ-1 proteins may aggregate and overwhelm the proteasome and this protein is oxidatively damaged in affected brain regions in PD and AD patients (111).

IMPLICATIONS

If the hypothesis (61, 64, 141) that proteasomal dysfunction is a major contributor to neurodegeneration is correct, several conclusions follow.

1. Agents that increase proteasome function, whether by relieving blockage or increasing transcription of genes encoding proteasome components, should be neuroprotective. We observed that overexpression of the antiapoptotic protein bcl-2 increases proteasome activity in cells (96), and it also delays cell death associated with the presence of mutant proteins, both *in vivo* and in cell culture (92, 95). Of course, these data do not prove that the bcl-2 is protecting by increasing proteasome activity, because this protein has multiple actions.
2. Because of their ability to block proliferation, cause apoptosis, and decrease NF-κB activation (which can reduce production of iNOS and proinflammatory cytokines), proteasome inhibitors are being extensively investigated for the treatment of cancer and chronic inflammatory diseases (1). They have also been proposed for use in stroke (163), and they can attenuate damage by suppressing inflammation and phagocyte recruitment (18, 122, 163). In rat cortical neurons, lactacystin blocked the cytotoxicity of β-amyloid (42). However, these studies were conducted over short time windows, and it is important to check that an initial protective effect is not followed by delayed neurotoxicity. Another area of interest is the possible use of proteasome inhibitors to protect against axonal degeneration (91).

However, when considering the therapeutic use of proteasomal inhibitors for the treatment of cancer or inflammatory disease, it is *essential* to ensure that the agents used do *not* cross the blood–brain barrier (61, 64, 103). Thus, infusion of lactacystin into the substantia nigra pars compacta of rats caused neurodegeneration and behavioral abnormalities (107). In a similar study (45), damage was selective for striatal dopamine cells and could be slowed by decreasing dopamine synthesis by using a tyrosine hydroxylase inhibitor, or

worsened by injecting L-Dopa or pargyline (to inhibit monoamine oxidase and increase dopamine levels). Treatment with proteasome inhibitors capable of crossing the blood–brain barrier caused adult rats to develop a progressive parkinsonian syndrome (104). As argued earlier, the possibility that many "natural" products and man-made chemicals [*e.g.*, dieldrin (148)] can interfere with the function of the ubiquitin–proteasome pathways and thus be potentially neurotoxic needs further investigation.

ABBREVIATIONS

AD, Alzheimer's disease; AGE, advanced glycation end product; ALS, amyotrophic lateral sclerosis; CJD, Creutzfeldt–Jakob disease; COX-2, cyclooxygenase-2; CSF, cerebrospinal fluid; CuZnSOD, copper- and zinc-containing superoxide dismutase; FALS, familial amyotrophic lateral sclerosis; GSH, reduced glutathione; GSSG, oxidized glutathione; HD, Huntington's disease; HNE, 4-hydroxynonenal; HPLC, high-performance liquid chromatography; Hsp, heat-shock protein; iNOS, inducible nitric oxide synthase; IRP-2, iron regulatory protein 2; αKGDH, α-ketoglutarate dehydrogenase; L-Dopa, L-dihydroxyphenylalanine; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase; 8OHdG, 8-hydroxy-2′-deoxyguanosine; 8OHG, 8-hydroxyguanine; PARP-1, poly(ADP-ribose)polymerase 1; PBN, *tert*-butyl-α-phenylnitrone; PD, Parkinson disease; RNS, reactive nitrogen species; SN, substantia nigra; UBB^{+1}, Alzheimer-associated variant ubiquitin; UCH L1, ubiquitin carboxy-terminal hydrolase L1.

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