

Altered Proteasomal Function in Sporadic Parkinson's Disease

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Parkinson's disease (PD) is characterized pathologically by preferential degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNc). Nigral cell death is accompanied by the accumulation of a wide range of poorly degraded proteins and the formation of proteinaceous inclusions (Lewy bodies) in dopaminergic neurons. Mutations in the genes encoding α -synuclein and two enzymes of the ubiquitin-proteasome system, parkin and ubiquitin C-terminal hydrolase L1, are associated with neurodegeneration in some familial forms of PD. We now show that, in comparison to age-matched controls, α -subunits (but not β -subunits) of 26/20S proteasomes are lost within dopaminergic neurons and 20S proteasomal enzymatic activities are impaired in the SNc in sporadic PD. In addition, while the levels of the PA700 proteasome activator are reduced in the SNc in PD, PA700 expression is increased in other brain regions such as the frontal cortex and striatum. We also found that levels of the PA28 proteasome activator are very low to almost undetectable in the SNc compared to other brain areas in both normal and PD subjects. These findings suggest that failure of the ubiquitin-proteasome system to adequately clear unwanted proteins may underlie vulnerability and degeneration of the SNc in both sporadic and familial PD.

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INTRODUCTION

The pathological hallmark of Parkinson's disease (PD) is selective degeneration of the dopamine-containing neurons in the substantia nigra pars compacta (SNc) (1). Nigral pathology is associated with the formation of intracellular inclusions (Lewy bodies) which contain a wide range of proteins, including α -synuclein, ubiquitin, neurofilament, and nitrated proteins (2–5). In addition, levels of oxidatively damaged proteins are

increased as evident from an elevation in the content of protein carbonyls and 4-hydroxynonenal (HNE) protein adducts in the SNc in PD (6, 7). There is also evidence to suggest that there is a general increase in protein aggregation in the substantia nigra in this disorder (8). These observations have led to the suggestion that impaired protein clearance and/or the overwhelming production of abnormal proteins play an important role in the neurodegenerative process occurring in PD (9).

The ubiquitin-proteasome system (UPS) is the primary biochemical pathway responsible for the degradation of normal and abnormal intracellular proteins, and its failure leads to protein accumulation and cell death (10, 11). Several studies have demonstrated that mutations in the genes encoding two enzymes of the UPS, parkin and ubiquitin C-terminal hydrolase L1 (UCH-L1), are associated with cases of familial PD (12, 13). In addition, mutations in the gene encoding α -synuclein are associated with nigral dopaminergic cell death with Lewy body formation in rare familial forms of PD (14–16). α -Synuclein is a 140-amino-acid protein and is a substrate for the UPS (12, 17–19). Mutations in α -synuclein cause the protein to misfold and aggregate, resist proteolysis, and inhibit proteasomal function (17, 20–22). Thus, the neurodegenerative process in α -synuclein-like familial PD could also relate to failure of the UPS to clear mutant α -synuclein and other proteins. Although these gene defects are not found in cases of sporadic PD which constitute the large majority of patients, they suggest that impairment of the UPS could also underlie nigral pathology in this disorder (9). Indeed, the accumulation of both nonubiquitinated and ubiquitinated proteins in the SNc and in Lewy bodies points to a primary defect in the proteasome, the central component of the UPS responsible for degrading the two groups of unwanted proteins (9, 10).

Proteasomes are multicatalytic proteases found in the cytoplasm, endoplasmic reticulum, perinuclear region, and nucleus of eukaryotic cells (10). The 20S proteasome is an assembly of two outer heptameric

rings of α -subunits and two inner heptameric rings of β -subunits (three of which host the different catalytic sites) that are stacked axially to form a hollow cylindrical structure in which proteolysis occurs (10). Binding of the multisubunit intracellular proteasome activators, PA700 and PA28, to the 20S proteasome form more active complexes known as the "26S proteasome" and the "activated 20S proteasome," respectively (10). While proteins that have been marked for proteolysis by labeling with a polyubiquitin chain are degraded by the 26S proteasome in an ATP-dependent manner, nonubiquitinated proteins and short peptides short-lived regulatory proteins are degraded by 20S proteasomes ATP independently (10).

In this study, we analyzed the integrity of 26/20S proteasomes, PA700 and PA28, and proteolytic enzyme activity in the brains of patients with sporadic PD and in age-matched control subjects.

MATERIALS AND METHODS

Materials

Aprotinin, leupeptin, pepstatin A, E-64, bestatin, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, Tween 20, BSA, Triton X-100, 3,3'-diaminobenzidine hydrochloride (DAB), and H_2O_2 were obtained from Sigma-Aldrich Corp. (St. Louis, MO). Protein assay reagents, Laemmli sample buffer, transfer buffer, polyvinylidene difluoride (PVDF), and polyacrylamide gels were obtained from Bio-Rad Laboratories (Hercules, CA). Horseradish peroxidase-conjugated secondary antibodies, biotinylated secondary antibodies, Vector SG chromogen, and the avidin:biotinylated peroxidase complex reagents were obtained from Vector Laboratories Inc. (Burlingame, CA). ECL detection reagents and Kodak X-omat films were obtained from Amersham Pharmacia Biotech Inc. (Piscataway, NJ). Suc-Leu-Leu-Val-Try-AMC, Z-Leu-Leu-Glu-AMC, and lactacystin were obtained from Calbiochem-Novabiochem Corp. (San Diego, CA). Boc-Leu-Arg-Arg-AMC was obtained from Bachem California Inc. (Torrance, CA). Polyclonal antibodies to the β -subunit and α -subunits of 26/20S proteasomes, PA700 (recognizes ATPase/non-ATPase subunits) and PA28 α , were obtained from Affiniti Research Products Ltd. (Exeter, UK) and Calbiochem-Novabiochem.

Postmortem Brain Tissue

Brain samples from 16 patients with clinical and pathological (loss of neuromelanin-pigmented neurons and Lewy bodies in the SNc) features of sporadic PD, and 13 age-matched control subjects without a clinical history or pathologic evidence of neurologic disease, were obtained from the Harvard Brain Tissue Resource Center (Belmont, MA), the Mount Sinai Brain

Bank (New York, NY), and the Parkinson's Disease Society Brain Research Center (London, UK). Sporadic PD and control cases were closely matched for age (PD, 73.8 ± 2.4 years; control, 75.5 ± 3.2 years; mean \pm SEM), sex (PD, 9 men/7 women; control, 7 men/6 women), and postmortem interval (PD, 22.5 ± 3.7 h; control, 25.1 ± 3.8 h; mean \pm SEM). Superior frontal cortex (Brodmann area 9), striatum (caudate-putamen-accumbens), hippocampal formation with parahippocampal gyrus, midbrain including the substantia nigra (the SNc was carefully dissected out), upper pons (level of locus ceruleus), and cerebellum with dentate gyrus were supplied as fresh frozen blocks for biochemical analyses from one hemisphere and as 10% buffered formalin-fixed coronal slices for immunohistochemical studies from the other hemisphere of the same brains.

Determination of the Levels of Proteasomal Components

PD and control brain tissues (unfixed) were retrieved from -70°C storage, homogenized (20 mg/ml) manually, and then homogenized by sonication in ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 1.0 mM, 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 80 μM aprotinin, 2 mM leupeptin, 1 mM pepstatin A, 1.5 mM E-64, 5 mM bestatin, 100 mM 4-(2-aminoethyl)benzenesulfonyl fluoride). Homogenates were centrifuged at $14,000g$ for 30 min at 4°C , supernatant was collected, and its protein concentration was determined by the Bradford method using Bio-Rad protein assay reagents with BSA as protein standard. Samples were mixed with an equal volume of Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 0.01% bromophenol blue) and boiled for 5 min, and then equal amounts (50 μg protein in 20 μl) of standard, PD, and control brain tissue per lane were loaded together onto the same polyacrylamide gels. Electrophoresis was performed in running buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 0.1% SDS). Proteins on gels were electrophoretically transferred to PVDF membranes in transfer buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine). Membranes were washed in TBS-T (20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 0.1% Tween 20), blocked in TBS-T containing 2.5% nonfat dry milk for 60 min, washed in TBS-T, and then incubated (overnight at 4°C) in TBS-T containing 2.5% nonfat dry milk and primary antibodies to the various proteasomal components (1:200). After the membranes were washed in TBS-T, they were incubated (60 min) in TBS-T containing 2.5% nonfat dry milk and horseradish peroxidase-conjugated secondary antibodies. Membranes were washed in TBS-T, incubated (1 min) in ECL detection reagents, dried, exposed to Kodak X-omat films in cassettes, and developed according to the manufacturer's instructions. Films were scanned into a computer and the NIH Image software (version 1.62)

used to quantify the density of each band. Specificity of immunoreactions on Western blots are evident from the appearance of bands at molecular weights (determined from the standard) for the various proteasomal components and the lack of immunostaining in the absence of primary antibodies (see Fig. 1). As an internal marker to ensure equal and consistent sample loading and electrophoresis, we monitored the levels of the 20S proteasome β -subunit. This has several advantages in that 20S proteasome α -subunits and β -subunits (and PA28) have similar kilodalton values and are stoichiometrically expressed and, from preliminary studies, we noted that the expression of β -subunits are similar in all brain regions studied with no significant difference between control and PD values (see Fig. 1).

Immunohistochemical Localization of Proteasomal Components

Buffered formalin-fixed PD and control brain tissues were cut to produce a series of 40- μ m-thick sections using a freezing microtome. Free-floating PD and control brain sections were immunostained in parallel using the following protocol. Sections were washed in 0.1 M PBS (pH 7.4) and endogenous peroxidase activity was quenched by incubation (10 min) in PBS containing 3% H₂O₂ and 10% methanol. After the sections were washed in PBS, nonspecific binding was blocked by incubation (60 min) in PBS containing 1% BSA/2% normal serum and 0.3% Triton X-100. Sections were washed in PBS and incubated (overnight at 4°C) in PBS containing 1% BSA/2% normal serum, 0.3% Triton X-100, and primary antibodies to the various proteasomal components (1:100). After washing in PBS, sections were incubated (60 min) in PBS containing 1% BSA/2% normal serum, 0.3% Triton X-100, and biotinylated secondary antibodies (1:200). Sections were washed in PBS and then incubated (60 min) in avidin:biotinylated peroxidase complex reagents (1:200). After washing in PBS, immunoreactions were visualized by incubating sections in 50 mM TBS (pH 7.4) containing Vector SG chromogen and 0.003% (w/w) H₂O₂ for 2 min to achieve optimal color intensity, and then the reaction was terminated by repeated washing in 50 mM TBS. Sections were dehydrated through 70, 95, and 100% ethanol; cleared in xylene; mounted onto gelatin-coated slides; and then covered with glass coverslips using DPX. The observation of a pattern of cellular and intracellular immunoreactivity consistent with previous studies and the absence staining without primary antibodies confirm the specificity of these immunoreactions (see Fig. 2).

Measurement of Proteasomal Activity

PD and control brain tissues (unfixed) were retrieved from -70°C storage and immediately homogenized by sonication in ice-cold 50 mM Tris-HCl (pH 7.5) con-

taining 1 mM EDTA. PD homogenates were kept on ice and used immediately for the fluorometric determination of 20S proteasomal enzymatic activity with 75 μ M Suc-Leu-Leu-Val-Try-AMC, Boc-Leu-Arg-Arg-AMC, and Z-Leu-Leu-Glu-AMC as substrates to measure the chymotrypsin-like, trypsin-like, and peptidyl glutamyl-peptide hydrolytic (PGPH) activities, respectively (23). 20S proteasomal enzymatic rates in PD and control tissues were measured in parallel and expressed as fluorescence units (FU)/min/ μ g brain tissue protein. Protein levels were determined as described above. Specificity of proteasomal assays was determined by the ability of 10 μ M lactacystin, a selective inhibitor of proteasomal function, to inhibit fluorescence change (23).

Statistical Analyses

We analyzed brains from 16 PD and 13 control subjects. Student's *t* test with Bonferroni correction to correct for multiple testing were used to determine differences in proteasomal enzymatic activity and subunit levels between PD and control tissues.

RESULTS

Levels of Proteasomal Components Are Altered in the PD Brain

To determine regional differences in the levels of proteasomal components in the brain of control and PD subjects, we undertook standard Western blot analyses with primary antibodies to the β -subunits and α -subunits of 26/20S proteasomes and the subunits of PA700 and PA28. These experiments revealed characteristic banding and molecular weights for the proteasomal components that were quantified by densitometry (Fig. 1a) (10, 24, 25).

In control brains, there was no difference in the content of the β -subunits (\approx 28 kDa) in the different brain regions studied, and these levels were not significantly altered in PD (Figs. 1a–1d). The amounts of α -subunits (\approx 29 kDa) varied in control brains brain regions (Figs. 1a–1d). In PD, there was a 40.2% ($P < 0.05$) reduction in the amount of α -subunits in the SNc (Figs. 1a–1d). Compared to controls, the levels of α -subunits were increased by 9.2% ($P > 0.05$) in the cerebral cortex and by 29.1% in the striatum ($P < 0.05$) in PD (Figs. 1a–1d).

The PA700 antibody employed recognizes conserved sequences in several ATPase/non-ATPase subunits of varying molecular weights in this proteasome activator (Fig. 1a) (10, 25). In control brain tissue, levels of PA700 subunits at the 81-, 75-, and 42-kDa bands were lower in the SNc than in the frontal cortex (Figs. 1a–1d, $P < 0.05$). In PD, there was a marked increase in the levels of subunits at the 81-, 75-, 52.5-, and 42-kDa

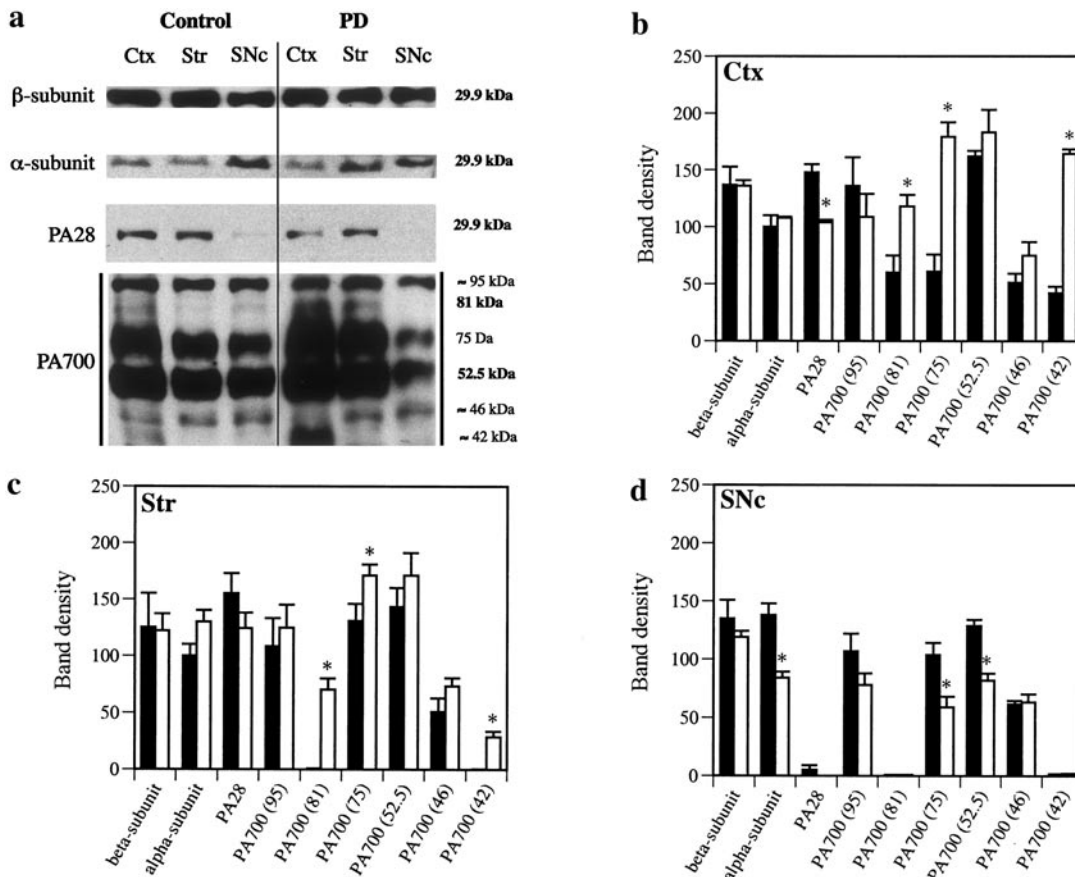


FIG. 1. Levels of proteasomal components in the frontal cortex, striatum, and SNc in sporadic PD and in age-matched controls. (a) Typical example of a Western blot analysis showing the separation and presence/absence of the β -subunits and α -subunits of 26/20S proteasomes and the subunits of PA28 and PA700. Approximate molecular weights for α -subunits, β -subunits, PA28, and PA700 bands are indicated on the right of the image with numbers in bold indicating almost exact weights compared to the standard. Note the reduction in the levels of 26/20S proteasome α -subunits in the SNc but not in the frontal cortex and striatum in PD. Also, components of PA700 fail to upregulate in the SNc although this occurred in the frontal cortex and striatum in PD compared to control. Finally, note that PA28 levels are low to almost absent in the SNc in both PD and control subjects. Appropriate controls for equal and consistent sample loading and electrophoresis are detailed under Materials and Methods section. (b–d) Quantification of the intensity of the bands on the Western blots depicted in Fig. 1a by densitometry using NIH Image 1.62. Numbers in parentheses represent approximate molecular weights. Results, presented as mean \pm SEM, were analyzed statistically using Student's *t* test with Bonferroni correction for multiple testing. **P* < 0.01, PD compared to control. Filled bar, control; Open bar, PD.

bands in the frontal cortex and/or the striatum compared to controls (Figs. 1a–1d). However, in the SNc, there was either no change (bands at 42, 46, and 95 kDa) or up to a 33% loss (bands at 52.5, 75, and 81 kDa) of PA700 subunits (Figs. 1a–1d). Since the molecular weights of PA700 components range from approximately 24.5 kDa (PSMD10) to 100 kDa (PSMD2), it is unclear whether some of the highest molecular weight bands in the Western blots represent single or dimerized subunits of PA700 (10).

Immunoreactivity for PA28 (\approx 28 kDa) was very low in the SNc compared to the frontal cortex and striatum in control subjects (Figs. 1a–1d). In PD brains, PA28 immunoreactivity was almost undetectable in the SNc and levels were reduced in the frontal cortex (24%, *P* < 0.05) and striatum (16%, *P* > 0.05) in comparison to controls (Figs. 1a–1d).

Selective Loss of 26/20S Proteasome α -Subunits in Dopaminergic Neurons of the SNc in PD

We immunostained sections from the formalin-fixed hemisphere of brains of PD cases and controls used for the Western blot studies. In control brain, cytoplasmic and nuclear immunoreactivity for the β -subunits (Figs. 2A and 2B) and α -subunits (Figs. 2E and 2F) of 26/20S proteasomes were found in neurons and glial cells in all brain regions, including dopaminergic neurons of the SNc and VTA as previously reported (26–28). In PD brains, immunoreactivity for the β -subunits of 26/20S proteasomes in the SNc and elsewhere was not significantly different compared to control tissues (Figs. 2A and 2B compared to Figs. 2C and 2D). In contrast, there was little or no immunoreactivity for the α -subunits of 26/20S proteasomes in the remaining dopami-

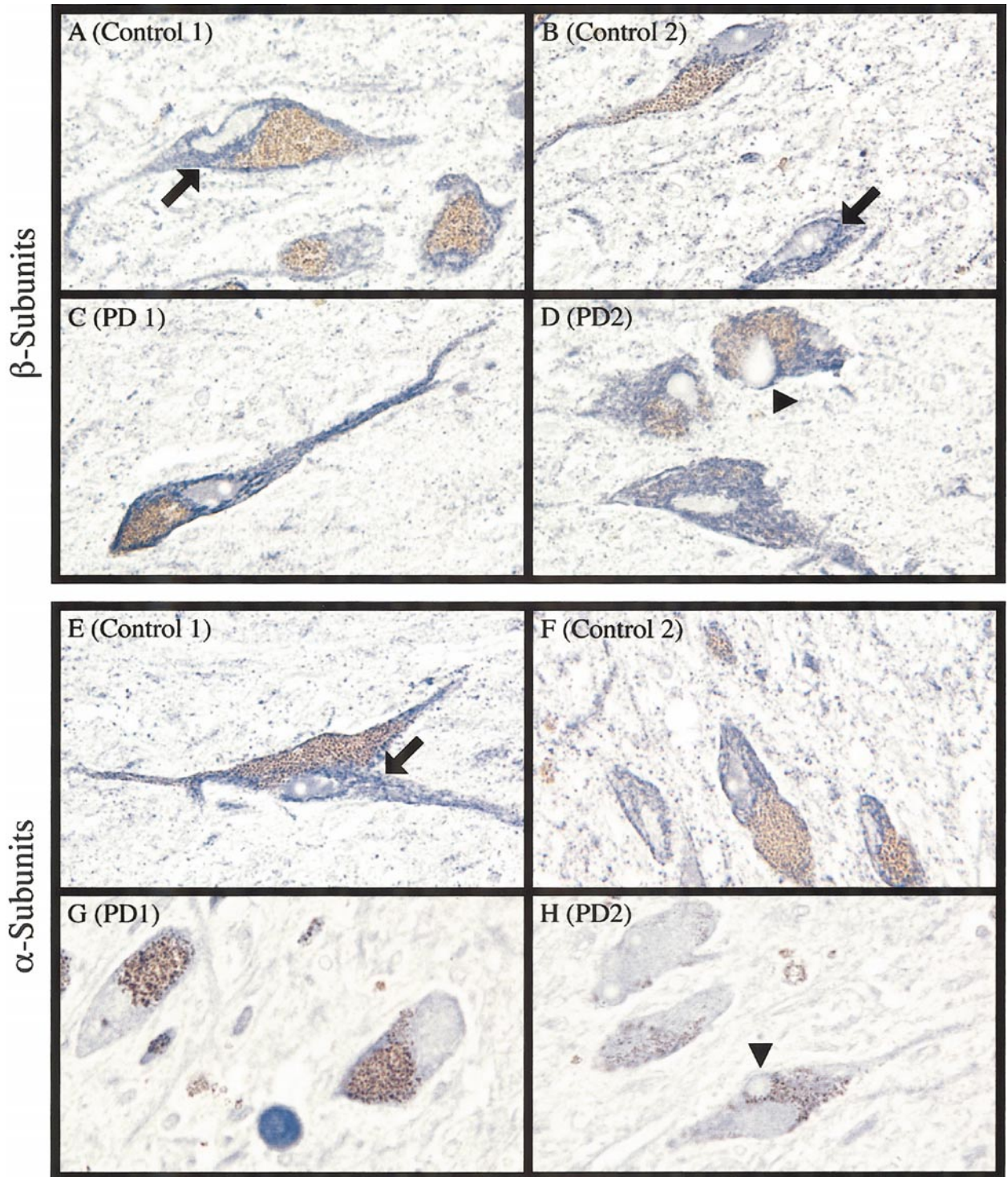


FIG. 2. Immunostaining of β -subunits and α -subunits of 26/20S proteasomes in the brains of sporadic PD and age-matched control subjects. Immunostained brain sections from two different control (Control 1 and Control 2) and two different PD (PD1 and PD2) subjects reveal proteasomal β - and α -subunits as a blue coloration (Vector SG chromogen, black arrows) in the cytoplasm, nucleus, and perinuclear region of neuronal and glial cells. Dopaminergic neurons are identified by the presence of brown neuromelanin deposits in their cytoplasm. Note that there is no change in staining for β -subunits in dopaminergic neurons in the SNc in PD (C and D) compared to controls (A and B). However, compared to controls (E and F), there is a loss of α -subunits in nigral dopaminergic neurons as shown by a marked reduction in blue cytoplasmic staining around the intracellular neuromelanin deposits in PD (G and H). The intensely stained blue circular structure in G resembles perinuclear accumulation of alpha-subunits to form an aggresome in a nondopaminergic cell and serves as a positive control for α -subunit in these sections. Black triangles in D and H indicate the presence of Lewy bodies in dopaminergic neurons in the SNc in PD. The results presented in this figure are representative of the other cases studied. $\times 600$ magnification.

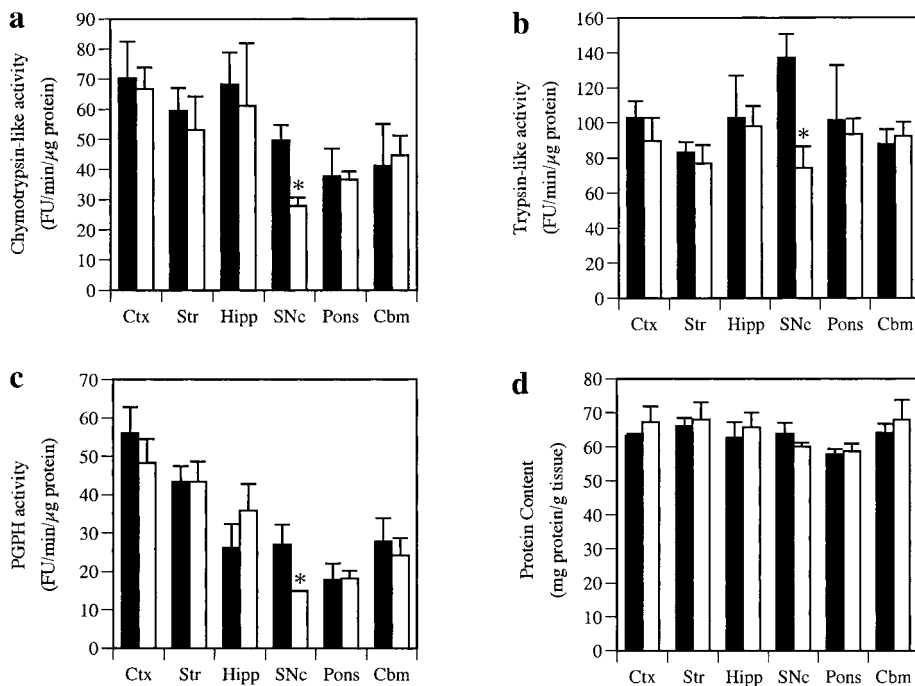


FIG. 3. Postmortem analyses of 20S proteasomal function in various brain regions in sporadic PD and age-matched controls. 20S proteasomal chymotrypsin-like, trypsin-like, and peptidyl glutamyl-peptide hydrolytic (PGPH) activities in homogenates of control (filled bar) and PD (open bar) brain tissues. Proteolytic activities (reaction rates) are expressed as fluorescence units (FU)/min/μg total tissue protein. Results, presented as mean \pm SEM ($n = 16$ PD cases; $n = 13$ control cases), were analyzed statistically using Student's t test with Bonferroni correction. * $P < 0.01$, PD compared to control. Ctx, frontal cortex; Str, striatum; Hipp, hippocampus; SNc, substantia nigra pars compacta; Cbm, cerebellum.

nergic neurons of the SNc as evident from the reduction or absence of cytoplasmic staining around intracellular neuromelanin deposits (Figs. 2E and 2F compared to Figs. 2G and 2H). Consistent with the Western blot analysis, PD and control brain tissue showed immunoreactivity for PA28 in the frontal cortex and striatum. However, PA28 staining in the SNc was very low with only prominent immunoreactivity in a few cells with the appearance of microglia and in the core of occasional Lewy bodies in PD (data not shown). In control tissues, PA700 immunoreactivity was present in glial and neuronal cells in all brain regions studied and staining was slightly reduced in PD (data not shown).

Proteolytic Activity of 20S Proteasomes are Selectively Impaired in the SNc in PD

To examine the proteolytic capacity of different brain regions, we determined the chymotrypsin-like, trypsin-like, and PGPH activities of 20S proteasomes which cleave proteins at hydrophobic, basic, and acidic residues, respectively (23). Proteolytic activities in homogenates from different brain regions of PD and control subjects are illustrated in Fig. 3. In homogenates from the SNc of PD brains, the chymotrypsin-like, trypsin-like, and PGPH activities were reduced by 43.9% ($P < 0.01$), 45.9% ($P < 0.01$), and 44.6% ($P < 0.01$), respec-

tively, compared to control values (Fig. 3). In contrast, each of the proteolytic activities of 20S proteasomes in the frontal cortex, striatum, hippocampus, pons, and cerebellum in PD brains were not significantly altered from control values (Fig. 3).

DISCUSSION

We report that the structure and function of 26/20S proteasomes are altered in the brain in sporadic PD. Specifically, in the SNc of PD patients there is a loss of α -subunits of 26/20S proteasomes within dopaminergic neurons and a reduction in each of the three proteasomal enzymatic activities. This was not observed in other brain regions. In addition, there was upregulation of PA700 in several brain regions, but not in the SNc. Finally, we note that levels of PA28 are very low in the SNc in comparison to other brain areas in both normal and PD subjects. These findings point to inadequate UPS activity and a protein handling dysfunction in sporadic PD.

Previous studies have shown that a reduction in the level of α -subunits causes the 26/20S proteasome complex to become unstable, prevents its normal assembly, and impairs proteolytic activity (10, 29). In addition, loss of α -subunits reduces binding of PA700 to the 20S proteasome thereby leading to a decrease in levels of

the 26 proteasome complex (30). Thus, our finding of reduced levels of α -subunits may be directly responsible for the decrease in 26/20S proteasomal enzymatic activity and failure of PA700 upregulation in the SNc in PD. Indeed, the small increase in expression of α -subunits in the cerebral cortex and striatum could underlie the marked upregulation in PA700 in these brain areas in PD. α -Subunits directly interact with some protein substrates and regulate their translocation and degradation within the 20S proteasome core (31, 32). Indeed, the degradation of oxidatively damaged proteins and α -synuclein, the levels of which are elevated in the SNc in PD, are thought to occur via the 20S proteasome core without an absolute requirement for PA700 or PA28 (6, 18, 32). Thus, a loss of 26/20S proteasome α -subunits could cause dysregulation as well as inhibition of proteasomal function leading to profound alterations in protein handling and consequently degeneration of the SNc in PD.

We found that the amounts of some PA700 subunits were lower in the SNc than in other regions of controls. In addition, PA28 levels were very low in the SNc in both control and PD subjects. The SNc normally has a higher level of basal protein oxidation than other brain regions which may relate to the oxidative metabolism of dopamine and the presence of high iron levels (33, 34). Therefore, even under normal circumstances, nigral dopaminergic neurons may be required to dispose of relatively high levels of oxidized proteins. Low PA28 levels and a reduced ability of PA700 to upregulate in response to stress could deprive nigral dopaminergic neurons of protective mechanisms for dealing with oxidatively damaged and other proteins (e.g., α -synuclein) which are increased in PD. These observations could account for why the SNc is particularly vulnerable to neurodegeneration. Interestingly, the expression of 20S proteasome α -subunits and the PA28 activator have been shown to decline with age in the mesencephalon of rats and this could also occur in humans (35). Thus, a lifetime accumulation of abnormal intracellular proteins due to oxidative damage and an increasingly incompetent UPS occurring in nigral dopaminergic neurons that do not turnover may explain why PD is an age-related disorder. In this regard, it is noteworthy that 5%–10% of clinically normal individuals over the age of 65 years have incidental Lewy bodies in dopaminergic neurons of the SNc (36).

The cellular specificity of proteasomal dysfunction in sporadic PD is not entirely clear since it is not possible to isolate and study the different cell types from post-mortem brain tissues. Although immunohistochemical analyses demonstrate a loss of 20S proteasome α -subunits within dopaminergic neurons, quantitative enzymatic assays and Western blot analyses reveal approximately 40% reduction in α -subunit levels and enzyme activity in homogenates of nigral tissues containing all cell types. Since dopaminergic neurons constitute <5%

of the total cellular population in the SNc, it is likely that proteasomal dysfunction also occurs to some extent in nondopaminergic in the SNc in sporadic PD. Indeed, such a concept is plausible given the possibility that the observed defects in 26/20S proteasomes could result from indiscriminate free radical-induced molecular damage in the SNc in sporadic PD.

The cause of defects in 26/20S proteasomes in sporadic PD is at present unknown, but could be a primary abnormality or occur as a result of another defect occurring in the neurodegenerative process. Mutations in one or several of the genes that encode for α -subunits or other components of 26/20S proteasomes may be responsible and need to be explored. Several widely distributed compounds are known to be potent inhibitors of proteasomal function and it is possible that these or other environmental factors that are toxic to the proteasome could play a role in the development of sporadic PD (37). Impairment of proteasomal function could also result from oxidant-mediated damage to proteins since oxidant defenses are reduced and levels of iron, peroxynitrite, end products of lipid peroxidation such as HNE and oxidized proteins are all elevated in the SNc in PD (38). Indeed, α -subunits of 26/20S proteasomes are selectively vulnerable to oxidative stress (30). Additionally, ATP is required for the assembly of the 20S proteasome and PA700 to form and maintain the 26S proteasome complex (25). Thus, inhibition of complex I activity, as occurs in the SNc in PD (39), could contribute to proteasomal dysfunction in this disorder.

Previous studies have shown that impairment of 26/20S proteasomal activity leads to the inhibition of peptide and protein hydrolysis, accumulation of ubiquitinated proteins, and cytotoxicity in neurons and cell lines (23, 40). We and others have recently shown that lactacystin-induced inhibition of 26/20S proteasomal function causes neuronal death with the formation of α -synuclein/ubiquitin-positive cytoplasmic inclusions in rat ventral mesencephalic dopaminergic neurons and PC12 cells (19, 41). Further, we recently demonstrated that stereotaxic infusion of lactacystin into the SNc of rats resulted in preferential degeneration of the dopaminergic nigrostriatal pathway with the cytoplasmic accumulation and aggregation of α -synuclein to form inclusion bodies and resultant motor dysfunction (42). Thus, our finding of altered 26/20S proteasomal structure and function, whether this occurs as a primary abnormality or the consequence of another defect in the neurodegenerative process, suggests that impaired protein handling could play a role in the initiation and/or progression of the nigral pathology that occur in sporadic PD.

In conclusion, we provide evidence for defects in proteasomes in sporadic PD and propose that failure of the UPS to degrade unwanted proteins could be a common etiopathogenic factor underlying protein accumulation

and/or Lewy body formation and ultimately neurodegeneration in both sporadic and familial PD.

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