

## Selective loss of 20S proteasome $\alpha$ -subunits in the substantia nigra pars compacta in Parkinson's disease

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### Abstract

The proteolytic activities of 26/20S proteasomes are impaired in the substantia nigra pars compacta (SNc) in sporadic Parkinson's disease (PD). In the present study, we examined the structural integrity of the proteasome by determining the levels of the  $\beta$ - and  $\alpha$ -subunits which together normally constitute the catalytic core of 26/20S proteasomes. Western blot analyzes and immunohistochemical staining revealed a major and selective loss of  $\alpha$ -subunits in dopaminergic neurons of the SNc but not in other brain regions in sporadic PD. This defect is known to cause the proteasome to become unstable and prevents its assembly with resultant impairment of enzymatic activity. Thus, structural and function defects in 26/20S proteasomes may underlie protein accumulation, formation of proteinaceous Lewy bodies and dopaminergic neuronal death in the SNc in sporadic PD. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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Parkinson's disease (PD) is a neurodegenerative disorder characterized clinically by rigidity, resting tremor, bradykinesia and postural abnormalities. The pathological hallmark of the illness is a relatively selective degeneration of the neuromelanin-pigmented dopaminergic neurons of the substantia nigra pars compacta (SNc) coupled with the formation of intracytoplasmic protein aggregates known as Lewy bodies [3]. The cause of neuronal death in PD is unknown, but the inability to degrade proteins due to failures in the ubiquitin-proteasome system (UPS) is implicated in rare familial forms of the disorder [9]. We now report that the UPS, through structural defects in 26/20S proteasomes, is also abnormal in SNc in sporadic PD and could contribute to the etiopathogenesis of this disorder.

The UPS is the primary pathway responsible for the degradation and clearance of used, damaged, mutant and mislocated intracellular proteins [19]. Thus, failure of the UPS leads to the accumulation of poorly degraded/unpro-

cessed proteins, inclusion body formation and consequent cell death [14]. Mutations in the genes encoding two different proteins necessary for normal UPS function, namely parkin and ubiquitin C-terminal hydrolase L1, are associated with the development of familial PD [6,15]. Similarly, mutations in the  $\alpha$ -synuclein gene, which cause the protein to misfold, aggregate, resist proteolysis and inhibit proteasomal function, underlie the development of rare familial forms of PD [12,17,18]. These gene defects have not been detected in sporadic cases which represent the vast majority of patients with PD. Nonetheless, protein handling dysfunction in the SNc in this disorder is evident from an increase in the levels of oxidatively damaged proteins, a general increase in protein aggregation and the accumulation of a wide range of poorly degraded proteins within Lewy bodies [1,3,7,16]. In addition, we recently showed that all three proteolytic activities of the 20S proteasome are impaired in the SNc in sporadic PD [8]. These observations suggest that protein handling dysfunction could underlie the neurodegenerative process that occurs in both familial and sporadic PD [9].

In this study, we assessed the structural integrity of 26/20S proteasomes in sporadic PD by determining the expres-

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sion of the  $\beta$ -subunits and  $\alpha$ -subunits which together normally constitute the proteolytic core of these enzyme complexes [19]. Brains were collected from patients with clinical and pathological (loss of neuromelanin-pigmented neurons with Lewy bodies in the SNc) findings of sporadic PD, and from control subjects without clinical or autopsy evidence of PD or other neurological illness. Cases were closely matched for age (PD,  $76.2 \pm 4.8$  years; control  $74.8 \pm 4.9$  years; mean  $\pm$  SEM;  $n = 6$  for each group) and post-mortem interval (PD,  $22.3 \pm 0.75$  h; control,  $21.1 \pm 3.8$  h; mean  $\pm$  SEM). Brain regions studied were the frontal cortex, striatum and SNc. Tissue was provided by the Harvard Brain Tissue Resource Center (Belmont, MA) and the Parkinson's Disease Society Brain Research Centre (London, UK). Primary polyclonal antibodies directed at components of 26/20S proteasomes were obtained from Affiniti Research Products Ltd (Exeter, UK) and Calbiochem (CA, USA).

Standard western blot analyzes using brain tissue homogenate supernatant (50  $\mu$ g total protein loaded per lane) revealed characteristic banding and molecular weights for 20S proteasomal  $\beta$ - and  $\alpha$ -subunits and were quantified by densitometry (Fig. 1A) [11,19]. In control brains, the content of  $\beta$ -subunits were similar in the various brain regions studied and these levels did not change significantly in PD (Fig. 1B). The content of  $\alpha$ -subunits were different in the various regions of control brains and this may reflect tissue-specific differences in the expression of this component which appears to play a pivotal role in the regulation of proteasomal function. In PD, there was a 39.1% reduction ( $P < 0.01$ ) in  $\alpha$ -subunit levels in the SNc but not elsewhere, compared to controls (Fig. 1B). Cellular localization of 20S proteasome components using immunohistochemical techniques revealed an almost total loss of  $\alpha$ -subunits (but not  $\beta$ -subunits) in dopaminergic neurons of the SNc but not in other areas examined in the PD brain (Fig. 2). The difference in the extent of  $\alpha$ -subunit loss revealed by the two methodologies likely relates to the fact that western blotting measures the total  $\alpha$ -subunit content from all cells in the SNc and would mask (effectively reduce) the greater loss of  $\alpha$ -subunits that occurs specifically in dopaminergic neurons. Consistent with this suggestion, intense and defined immunoreactivity for  $\alpha$ -subunits can be seen in non-dopaminergic cells throughout the SNc in PD (Fig. 2).

The catalytic sites of 26/20S proteasomes are located on the  $\beta$ -subunits which appear to be preserved in the SNc in sporadic PD [19]. However, a loss of  $\alpha$ -subunits is known to cause 26/20S proteasomes to become unstable and prevents their normal assembly which result in impaired functional activity [4,19]. This defect could therefore underlie impaired proteasomal function, protein accumulation and neurodegeneration that occur in the SNc in sporadic PD [8]. Indeed, we and other have recently shown that lactacystin, a selective proteasomal inhibitor, causes concentration-dependent cell death with the formation of Lewy body-like cytoplasmic inclusions containing  $\alpha$ -synuclein and

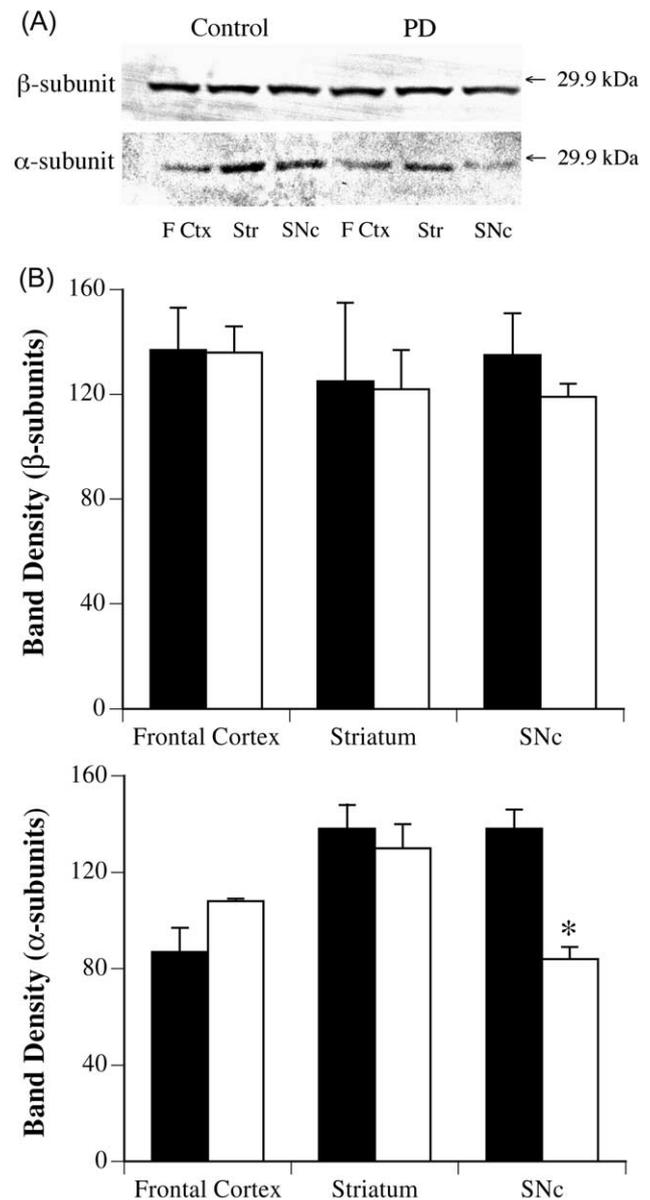


Fig. 1. 20S proteasome  $\beta$ - and  $\alpha$ -subunit levels in PD and control brains. (A) Typical example of a Western blot analysis showing the presence and absence of proteasomal components in brain tissue. (B) Quantification by densitometry using the NIH Image 1.62 software demonstrated a 39.1% loss of  $\alpha$ -subunits in the SNc but not in other regions of the brain in PD. Results, presented as mean  $\pm$  SEM ( $n = 6$ ), were analyzed statistically using the Student's  $t$ -test. \* $P < 0.01$ . Filled bars, control; and open bars, PD.

ubiquitin in cultured dopaminergic neurons and PC12 cells [10,13]. The basis for the loss of 20S proteasome  $\alpha$ -subunits in nigral dopaminergic neurons in sporadic PD is not known, but could relate to gene mutations, translational defects or results from toxic processes such as oxidative stress. Indeed,  $\alpha$ -subunits are particularly vulnerable to oxidative damage, and free radical-mediated attack on proteins and other macromolecules occur in the SNc in PD [2,5].

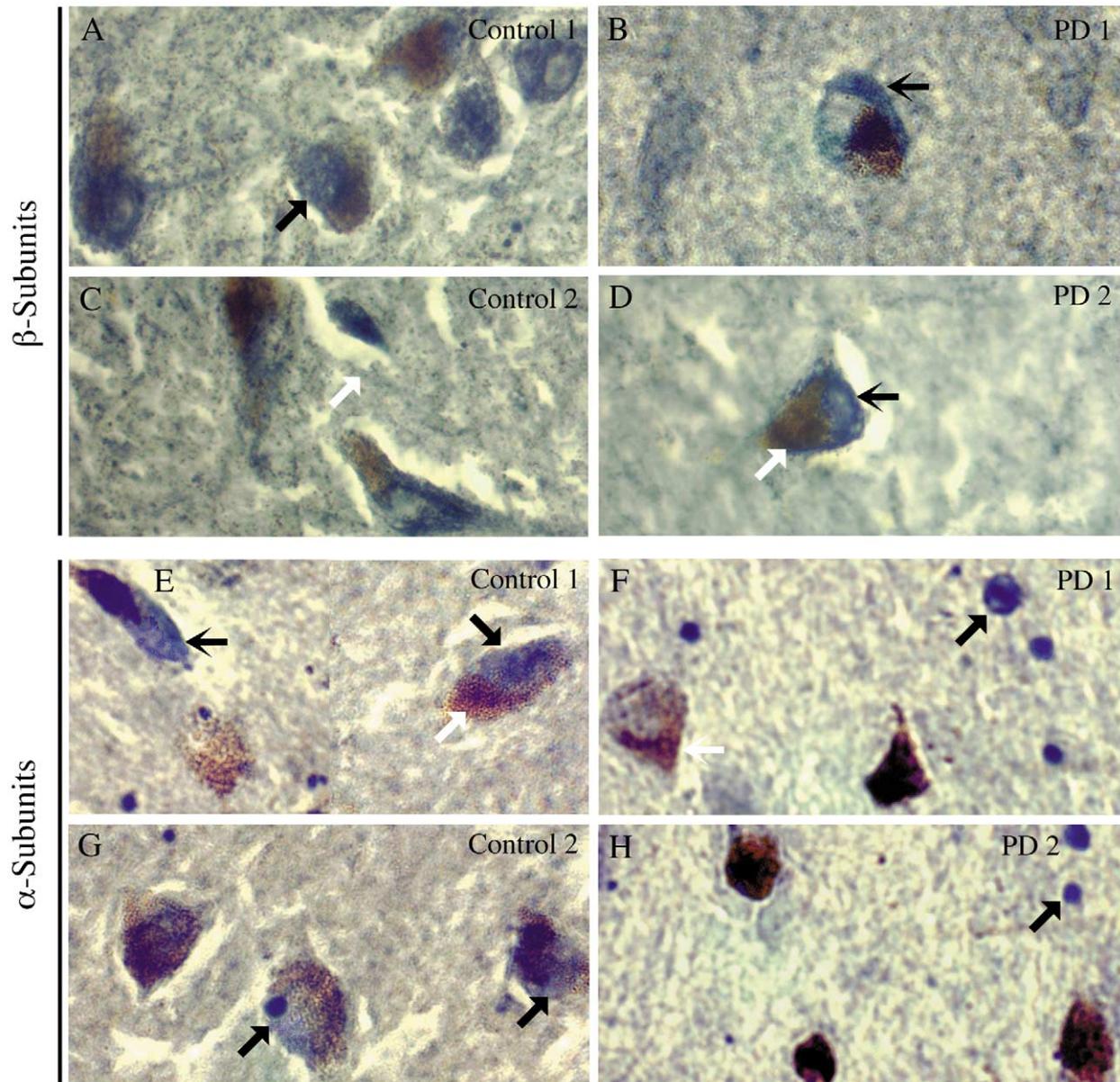


Fig. 2. Cellular localization of  $\beta$ - and  $\alpha$ -subunits of the 20S proteasome in PD and control brains. Immunostained brain sections from PD and control subjects reveal proteasomal  $\beta$ - and  $\alpha$ -subunits as a blue coloration in the cytoplasm, nucleus and perinuclear region (black arrows) of neuronal and glial cells. Dopaminergic neurons are clearly identified by the presence of brown neuromelanin deposits (white arrows) in their cytoplasm. Note that there is a loss of staining for  $\alpha$ -subunits in dopaminergic neurons in PD (panels F and H) versus controls (panels E and G), but no change in staining for the  $\beta$ -subunits (panels B and D compared to panels A and C). The highly stained blue circular structures (black arrows in panels F and H) are aggresome/corpora amylacea-like perinuclear concentration of  $\alpha$ -subunits in non-pigmented dopaminergic cells in the PD nigra and serve as a positive control for  $\alpha$ -subunit staining in these sections [20]. The results presented were obtained from brain sections from two different PD patients (PD1 and PD2) and from two different control subjects (Controls 1 and 2) and are representative of all cases studied.

In conclusion, we show a selective loss of 20S proteasome  $\alpha$ -subunits in dopaminergic neurons of the SNc in sporadic PD. This finding suggests that failure of the UPS to clear unwanted proteins could be a common etiopathogenic factor in sporadic and the various familial forms of PD.

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