INTRAPALLIDAL LIPOPOLYSACCHARIDE INJECTION INCREASES IRON AND FERRITIN LEVELS IN GLIA OF THE RAT SUBSTANTIA NIGRA AND INDUCES LOCOMOTOR DEFICITS

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Abstract—Increasing evidence suggests that abnormal iron handling may be involved in the pathogenesis of Parkinson’s disease. The present study investigates the role of iron and the iron-storage protein ferritin in inflammation-induced degeneration of dopaminergic neurons of the substantia nigra pars compacta. Injection of lipopolysaccharide into the globus pallidus of young and middle-aged rats substantially decreased tyrosine hydroxylase immunostaining in substantia nigra pars compacta four weeks after injection. Loss of tyrosine hydroxylase expression was accompanied by increased iron and ferritin levels in glial cells of the substantia nigra pars reticulata. Despite greater increases in nigral iron levels, ferritin induction was less pronounced in older rats, suggesting the regulation of ferritin was compromised with age. Automated movement tracking analyses showed that young rats recovered from LPS-induced locomotor deficits within four weeks, yet older rats failed to improve on measures of speed and total distance moved. Intrapallidal lipopolysaccharide injection also increased expression of α-synuclein and ubiquitin in tyrosine hydroxylase-positive neurons of the substantia nigra pars compacta. These results suggest that pallidal inflammation significantly increases stress on dopamine-containing neurons in the substantia nigra pars compacta. Alterations in nigral iron levels and protein handling may increase the vulnerability of nigral neurons to degenerative processes. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: globus pallidus, inflammation, oxidative stress, microglia, alpha-synuclein, ubiquitin.

In Parkinson’s disease, clinical symptoms including tremor, rigidity, and bradykinesia result from the loss of dopamine-containing neurons in the substantia nigra pars compacta. Many interacting pathological processes appear to contribute to neuronal degeneration in the disease. Microglial activation and increased cytokine levels mark inflammation in substantia nigra of Parkinson’s disease patients (McGeer et al., 1988a,b; Boka et al., 1994; Mogi et al., 1994a,b), and observations of increased superoxide dismutase activity, decreased levels of reduced glutathione, and decreased catalase activity in substantia nigra provide evidence for oxidative stress (Riederer et al., 1989; Saggau et al., 1989; Gerlach et al., 1994). In addition, a shift in Fe²⁺/Fe³⁺ ratios and overall increases in total iron content have been described in substantia nigra of parkinsonian brain and related to the severity of the disease (Dexter et al., 1987; Sofic et al., 1988; Riederer et al., 1989; Gotz et al., 2004). High concentrations of iron in the basal ganglia, particularly in substantia nigra and globus pallidus, may contribute to neurodegeneration by exacerbating oxidative stress and promoting aggregation of α-synuclein (Sofic et al., 1988; Dexter et al., 1989; Griffiths and Crossman, 1993; Hashimoto et al., 1999; Ostrerova-Golts et al., 2000; Golts et al., 2002; Gotz et al., 2004).

Recently, studies in rats have demonstrated that inflammation induced by intranigral or perinigral injection of lipopolysaccharide (LPS) replicates characteristics of Parkinson’s disease, including selective loss of nigral dopaminergic neurons and extensive activation of microglia (Bing et al., 1998; Castano et al., 1998; Herrera et al., 2000; Gao et al., 2002; Arimoto and Bing, 2003). To further study the role of inflammation in nigrostriatal pathophysiology, our laboratory has injected LPS into rat globus pallidus. The globus pallidus is a major integrative nucleus within the basal ganglia, with neurons projecting to striatum, subthalamic nucleus, entopeduncular nucleus, and substantia nigra pars reticulata (Blandini et al., 2000; Parent et al., 2000). Thus, the globus pallidus is positioned to influence the nigrostriatal pathway and function of the basal ganglia as a whole. We have demonstrated that injection of LPS into the globus pallidus decreases immunostaining for tyrosine hydroxylase (TH) in the substantia nigra pars compacta, suggesting that inflammation in globus pallidus can affect viability of nigral dopaminergic neurons.

The globus pallidus contains the greatest concentration of iron within the adult human brain and thus may be a potent source of oxidative stress (Double et al., 2000; Gotz et al., 2004). The current study investigates the role of iron in substantia nigra vulnerability to intrapallidal LPS injection. Following LPS administration, immunoreactivity for the iron storage protein ferritin was increased in the substantia nigra of young and middle-aged rats. In addition, intrapallidal LPS increased iron levels in glial cells of the substantia nigra. Finally, injection of LPS increased...
α-synuclein and ubiquitin levels in dopaminergic neurons of the substantia nigra pars compacta of middle-aged animals. These results demonstrate that iron and protein handling in substantia nigra are altered following inflammation in globus pallidus, with potentially damaging effects on nigral dopaminergic neurons.

**EXPERIMENTAL PROCEDURES**

Male Fischer 344 rats 3 months of age (young, n=20) and 16 months of age (middle-aged, n=20) received bilateral injections of LPS (Sigma; LPS Escherichia coli 0111:B4; L3022; Lot #072K4093; 10 μg/4 μl/site) or saline (4 μl/site) into globus pallidus at coordinates: -1.3 mm from bregma, ±3.2 mm lateral to midline, and 6.5 mm from dura (n=5 per age/treatment group). Four weeks after LPS injection, animals were deeply anesthetized with pentobarbital (100 mg/kg) and decapitated. The substantia nigra was dissected out, and protein was extracted for Western blot analysis. The remaining 20 animals (10 from each group) were deeply anesthetized with pentobarbital (100 mg/kg) prior to transcardial perfusion with PBS followed by 4% paraformaldehyde. All animal procedures were performed in accordance with the National Institutes of Health guidelines and approved by the University of Kentucky Institutional Animal Care and Use Committee. All measures were taken to minimize animal pain or discomfort.

**Immunohistochemistry**

Perfused brains were sectioned at 30 μm on a sliding microtome into free-floating tissue sections. Every sixth section from the region containing substantia nigra was incubated overnight at 4 °C with a primary antibody against OX-42 (monoclonal, 1:4000, PharMingen, San Diego, CA, USA), TH (monoclonal, 1:1000, Chemicon, Temecula, CA, USA), or ferritin (rabbit polyclonal, 1:500, Zymed Laboratories, San Francisco, CA, USA). After washes and incubation with an appropriate secondary antibody (1:4000, Vector Laboratories, Burlingame, CA, USA), nonreactive cells were visualized by the avidin–biotin immunoperoxidase method (ABC Kits, Vector Laboratory, Burlingame, CA, USA) with a primary antibody against OX-42 (monoclonal, 1:4000, Sigma) or rabbit anti-ubiquitin (1:1000, Sigma) primary antibody overnight at 4 °C followed by incubation with Alexa Fluor 488 goat-anti-rabbit secondary antibody (1:1000, Molecular Probes Inc.) for 1 h. The sections were subsequently incubated with mouse anti-TH primary antibody (1:1000 Chemicon Inc.) overnight at 4 °C, followed by incubation for 1 h in Alexa Fluor 568 goat-anti-mouse IgG secondary antibody (1:1000, Molecular Probes Inc.).

Colocalization of TH with α-synuclein or ubiquitin was also examined. Sections were incubated with rabbit anti-α-synuclein (1:500, Sigma) or rabbit anti-ubiquitin (1:1000, Sigma) primary antibody overnight at 4 °C followed by incubation in Alexa Fluor 488 goat anti-rabbit secondary antibody (1:1000, Molecular Probes Inc.) for 1 h. The sections were subsequently incubated with mouse anti-TH primary antibody (1:1000 Chemicon Inc.) overnight at 4 °C, followed by incubation for 1 h in Alexa Fluor 568 goat-anti-mouse IgG secondary antibody (1:1000, Molecular Probes Inc.).

Fluorescent preparations were examined using the Leica TCS SP laser scanning confocal imaging system (Leica Microsystems, Inc., Bannockburn, IL, USA). Images were viewed on a Leica DM RXE upright microscope. Images were acquired simultaneously for both fluorophores [Alexa Fluor 488 (green) and Alexa Fluor 568 (red)] using argon and krypton lasers, respectively. Regions exhibiting colocalization of the red and green emitters produced yellow fluorescence.

**Perl’s iron stain**

For Perl’s staining, sections were processed through a series of graded alcohols, into xylene, and rehydrated back to water. Sections were incubated in a 1:1 solution of 2% HCl and potassium ferrocyanide (Sigma) for 30 min and rinsed in water. Sections were counterstained with Neutral Red, dehydrated in increasing concentrations of ethanol, cleared in xylene, and mounted on slides.

**Automated movement tracking**

Spatiotemporal measures of animal movements were recorded in an open field (40×40 cm) using a video-based track-tracing system (EthoVision®, version 2.3, Noldus Information Technology, Wageningen, the Netherlands). Animals underwent habituation in the open field for 30 min on the day prior to the first sampling and for 10 min before each sampling period. Video images were recorded by an 8 mm Sony digital camera and played back with a Sony EV-90000 digital videocassette recorder coupled to a Dell Dimension Deskpro 8100 computer. Ethovision software allowed measurement of movement distance and speed by automatically track-tracing animal displacement in a defined arena. Images of these displacements were acquired at a rate of six samples/s for a 30 min observation period. Video records were analyzed for total distance traveled (cm) and movement speed (cm/s). Data were analyzed by two-way ANOVA with repeated measures over time. Post hoc comparisons were made using the Bonferroni-Dunn test.

Data were analyzed for significant effects by ANOVA using StatView 5.0 (SAS Institute, Inc., Cary, NC, USA). Post hoc comparisons were made using the Bonferroni-Dunn test, with significance set at P<0.05. Data are presented as mean band density±standard error.

**Immunofluorescence and laser scanning confocal microscopy**

For ferritin co-localization with glial fibrillary acidic protein (GFAP), OX-42, or TH, free-floating sections were incubated with mouse anti-GFAP (1:1000, Chemicon Inc.), mouse anti-OX42 (1:2000, PharMingen), or mouse anti-TH (1:1000, Chemicon Inc.) primary antibody overnight at 4 °C, followed by incubation for 1 h in Alexa Fluor 568 goat anti-mouse IgG secondary antibody (1:1000, Molecular Probes Inc., Eugene, OR, USA). The sections were then incubated with rabbit anti-ferritin serum (1:500, Molecular Probes, Inc.) overnight at 4 °C followed by incubation with Alexa Fluor 488 goat anti-rabbit secondary antibody (1:1000, Molecular Probes Inc.) for 1 h. Sections were mounted on slides with ProLong mounting medium (Molecular Probes, Inc.) in preparation for confocal microscopy.

For Perl’s staining, sections were processed through a series of graded alcohols, into xylene, and rehydrated back to water. Sections were incubated in a 1:1 solution of 2% HCl and potassium ferrocyanide (Sigma) for 30 min and rinsed in water. Sections were counterstained with Neutral Red, dehydrated in increasing concentrations of ethanol, cleared in xylene, and mounted on slides.
with significance set at $P<0.05$. Data are presented as mean distance or speed ± standard error.

**RESULTS**

**Microglial activation in globus pallidus**

Intrapallidal LPS injection increased expression of OX-42, a marker of microglial activation, in globus pallidus of young (3 months) and middle-aged (16 months) rats four weeks after injection (Fig. 1). OX-42 staining thus demonstrates sustained inflammation in globus pallidus following LPS infusion. Fig. 1 also shows that LPS diffuses only ~1 mm from the site of injection. Injections were administered approximately 3.6 mm anterior to substantia nigra, indicating that LPS would not have passed to substantia nigra by diffusion.

**TH immunoreactivity in substantia nigra pars compacta**

Intrapallidal LPS injection led to a significant and age-dependent loss of TH expression (Fig. 2). Four weeks following injection, both young and middle-aged animals exhibited a loss of TH immunostaining in dopaminergic neurons of the substantia nigra pars compacta, which was more pronounced in older animals (Fig. 2A). Following Western blot analysis of TH levels in the whole substantia nigra, two-way ANOVA indicates a significant main effect of injection [$F(1, 16)=64.53; P<0.05$]. Post hoc comparisons indicated that injection of LPS decreased TH levels in young and middle-aged subjects. A significant age/injection interaction effect on TH levels was also observed [$F(1, 16)=7.72; P<0.05$]. Older rats injected with LPS experienced more than twice the amount of TH loss as compared with younger animals (Fig. 2B).

**Ferritin expression in substantia nigra**

Intrapallidal injection of LPS increased immunoreactivity for ferritin in the substantia nigra of both young and middle-aged animals (Fig. 3). Immunohistochemistry indicated

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**Fig. 1.** Intrapallidal LPS injection increases microglial activation in globus pallidus. A representative photomicrograph shows OX-42 immunoreactivity in globus pallidus four weeks after LPS injection. (GP, globus pallidus; ST, striatum; 3rd V, 3rd ventricle; scale bar = 1 mm.)

**Fig. 2.** Intrapallidal LPS injection differentially decreases TH immunoreactivity in the substantia nigra pars compacta of young and middle-aged rats. (A) Immunocytochemistry demonstrates a greater loss of TH immunostaining in substantia nigra pars compacta (arrows) of middle-aged rats as compared with young rats four weeks after intrapallidal LPS injection. (B) Western blot analysis indicates significant loss of TH immunoreactivity in whole substantia nigra of young and middle-aged rats four weeks after intrapallidal LPS injection. The LPS-induced loss of TH immunoreactivity is significantly greater in older animals. [*] $P<0.05$ compared with saline within the same age group (effect of injection); [#] $P<0.05$ compared with young animals within the LPS treatment group (effect of age).
that increases were mainly confined to the substantia nigra pars reticulata. Western blot analysis of the whole substantia nigra showed that LPS treatment significantly increased ferritin immunoreactivity in young \( F(1, 6)=8.81; P<0.05 \) and middle-aged \( F(1, 6)=35.06; P<0.05 \) rats.

Confocal microscopy demonstrated that following LPS injection, ferritin expression in substantia nigra pars reticulata was strongly colocalized with OX-42. Little overlap with GFAP, a marker for astrocytes, was observed (Fig. 4). These data indicate that the majority of LPS-induced fer-

Fig. 3. Intrapallidal LPS injection increases ferritin expression in substantia nigra of young and middle-aged animals. Ferritin immunoreactivity was increased in substantia nigra pars reticulata of LPS-treated rats but not altered in substantia nigra pars compacta (panels A–D). Scale bar=500 μm. Inset boxes show higher magnification \((40×)\) of substantia nigra pars reticulata. Western blot analysis demonstrates that increased ferritin expression is significant in substantia nigra of young and middle-aged LPS-treated rats (one-way ANOVA, \( P<0.05 \)).

Fig. 4. Ferritin is expressed in microglial cells of the substantia nigra pars reticulata four weeks following intrapallidal LPS injection in middle-aged rats (upper panels). Double-labeling for GFAP or OX-42 (red) with ferritin (green) shows that immunostaining for ferritin is mainly co-localized (yellow) with OX-42, a microglial marker. In substantia nigra pars compacta of middle-aged rats (lower panels), double-labeling for TH (red) with ferritin (green) indicates little LPS-induced ferritin expression in TH-positive neurons. Scale bar=40 μm.
ritin expression in substantia nigra pars reticulata occurs in microglial or macrophage-like cells. In substantia nigra pars compacta, ferritin colocalization with TH-positive neurons was evident, but less pronounced than in microglia (Fig. 4).

Iron levels in substantia nigra
Perl’s iron stain revealed very little positive staining for iron in substantia nigra of young, saline-injected rats (Fig. 5A). Iron levels were visibly higher in substantia nigra of middle-aged, saline-injected rats (Fig. 5C). Treatment with LPS increased iron levels in both young and middle-aged rats, with more pronounced increases in older animals (Figs. 5B and 5D). In general, Perl’s stain for iron was localized predominantly to small, glial-like cells in substantia nigra pars reticulata (Fig. 5B–D).

Alpha-synuclein and ubiquitin expression in dopaminergic neurons of the substantia nigra pars compacta
Confocal microscopy demonstrated colocalization of α-synuclein and ubiquitin with TH-positive neurons in substantia nigra pars compacta of LPS-injected, middle-aged rats (Fig. 6). We have found a clear and marked increase of α-synuclein and ubiquitin immunoreactivity present in the SNpc in four of six middle-aged animals and an attenuated response in the other two rats. However, immunostaining for α-synuclein and ubiquitin was not visible in saline-treated animals or young animals (data not shown).

Recovery from inflammation-induced locomotor deficits is age-dependent
Activity levels were quantified in young and middle-aged rats before injection and at 1, 2, 3, and 4 weeks post-surgery (Fig. 7). Analysis of movement speed by two-way ANOVA with repeated measures over time demonstrated a significant injection/age interaction [$F(4, 96)=3.44; P<0.05$]. The Bonferroni-Dunn test indicated that middle-aged, LPS-injected rats moved more slowly at weeks 1, 3, and 4 as compared with pre-surgery movement speed (Fig. 7A). In contrast, the movement speed of young, LPS-injected rats was significantly reduced only for the first two weeks post-injection and then showed recovery toward saline-treated control values. Movement speed remained relatively stable in the saline-treated controls.

Analysis of total distance moved (Fig. 7B) by two-way ANOVA with repeated measures over time indicates a significant injection/age interaction [$F(4, 96)=3.5; P<0.05$]. The Bonferroni-Dunn test was used to compare all time points within each age/injection group, with significance set at $P<0.005$. In middle-aged rats injected with LPS, a significant reduction in total distance moved was observed at 1, 2, 3, and 4 weeks following injection, as compared with the pre-surgery time point. Young, LPS-treated rats displayed decreases in distance moved at 1, 2, and 3 weeks post-injection, yet by four weeks they had recovered to pre-surgery levels.

DISCUSSION
The present study demonstrates that LPS injection into globus pallidus produces a localized pallidal inflammation
and changes in the substantia nigra similar to some aspects of Parkinson’s disease. Most notably, intrapallidal injection of LPS led to a significant age-dependent loss of TH, the rate-limiting enzyme in dopamine synthesis. Older rats experienced more than twice the amount of TH immunoreactivity loss as compared with younger rats, which was associated with significant deficits in locomotor activity from which older rats did not recover over the four week test period. Intrapallidal LPS increased iron content and ferritin levels in substantia nigra, indicating that inflammation in globus pallidus can influence nigral iron handling. In addition, accumulation of α-synuclein and ubiquitin was observed in dopaminergic neurons of the substantia nigra pars compacta, suggesting that inflammation in the globus pallidus can induce protein mishandling in substantia nigra pars compacta. Finally, decreased immunostaining for TH in substantia nigra suggests a moderate injury response of dopaminergic neurons to globus pallidus inflammation.

Fig. 6. Intrapallidal LPS injection induces α-synuclein and ubiquitin expression in dopaminergic neurons of the substantia nigra pars compacta in middle-aged rats. Upper panels: double-labeling for TH (red) and α-synuclein (green) in substantia nigra pars compacta 4 weeks after LPS injection demonstrates accumulation of α-synuclein in TH-immunoreactive neurons (yellow). Alpha-synuclein expression appears in the cytoplasm of dopaminergic neurons as small patches or inclusions. Lower panels: double-labeling for TH (red) and ubiquitin (green) in substantia nigra pars compacta 4 weeks after LPS injection demonstrates abundant levels of ubiquitin in TH-immunoreactive neurons (yellow). The distribution of ubiquitin differs from that of α-synuclein. Ubiquitin expression is more diffuse in the cytoplasm of TH-immunoreactive neurons. Scale bar~20 μm.

Fig. 7. Locomotor deficits following intrapallidal LPS injection were more severe in older rats. (A) Movement speed (cm/s). Compared with pre-surgery measures, middle-aged LPS-injected rats moved more slowly at weeks 1, 3, and 4 post-surgery. In contrast, movement speed of young, LPS-injected subjects was significantly depressed in the first two weeks following LPS administration and showed evidence of recovery beginning by week 2. The values for the saline controls were relatively stable. (B) Total distance traveled (cm) per 30 min observation period. Compared with the pre-surgery time point, distance traveled was significantly reduced in the middle-aged LPS-treated animals at weeks 1, 2, 3, and 4 post-surgery. Young LPS-treated animals also showed reductions in travel at weeks 1, 2, and 3 post-surgery, yet had recovered to baseline levels by week 4. Compared with the pre-surgery time point, there were no significant effects of saline injection on young or aged rats. (* P<0.005 within age/injection group compared with pre-surgery).
Decreased ferritin expression may impair iron handling with age

Ferritin is one of the principal iron storage proteins in the body (Harrison and Arosio, 1996). By maintaining iron in a nonreactive form, ferritin protects cells from the generation of reactive oxygen species that can occur in the presence of free iron. However, in Parkinson’s disease, impaired regulation of iron content and ferritin are proposed to contribute to oxidative stress and the degeneration of nigral dopaminergic neurons (Dexter et al., 1991; Gerlach et al., 1994).

Ferritin biosynthesis is positively regulated by iron (Casey et al., 1988), suggesting that increased nigral ferritin levels following intrapallidal LPS injection are attributable to increased iron levels in the same region (Figs. 3 and 5). However, it is interesting to note that while ferritin levels increased by approximately 100% in young, LPS-injected rats, ferritin levels only increased by approximately 50% in older rats (Fig. 3). This attenuated induction of ferritin in older rats, coupled with the relative increase in iron content in substantia nigra pars reticulata following LPS injection in older rats (Fig. 5), suggests that iron handling may be impaired in middle-aged rats challenged with LPS.

Impaired ferritin regulation in older rats may be related to enhanced inflammation following LPS administration. For instance, LPS induces microglial activation and expression of inducible nitric oxide synthase (iNOS) (Arimoto and Bing, 2003), and we have observed that LPS-induced activation of microglial cells is more robust in middle-aged rats (unpublished observation). Glial activation and iNOS expression are also increased with age following cortical stab injury in rats (Kyrkanides et al., 2001), and in glial cell cultures, mitogen-stimulated nitric oxide (NO) production increases with age of donor (Yu et al., 2002). These reports suggest that the inflammatory response and production of NO are amplified with age. NO inhibits ferritin expression by increasing iron regulatory protein 1 (IRP1) binding to 5’ untranslated iron-responsive elements on ferritin mRNA (Henitze and Kuhn, 1996; Chenais et al., 2002). Thus, greater levels of NO released by activated microglia might differentially decrease ferritin biosynthesis in older animals. A similar scenario has been described in Parkinson’s disease, where IRP1 binding and ferritin levels are unaltered despite increased iron content in substantia nigra pars compacta (Faucheux et al., 2002).

It is widely accepted that iron levels increase in the substantia nigra of patients with Parkinson’s disease (Sofic et al., 1988; Riederer et al., 1989; Griffiths et al., 1999), and altered iron handling has been proposed to contribute to the pathogenesis of the disease (Dexter et al., 1991; Gerlach et al., 1994). However, studies measuring ferritin content have reported inconsistent findings. Some studies report reduced or unchanged ferritin levels in substantia nigra of Parkinson’s disease patients (Dexter et al., 1990; Mann et al., 1994), while others have described increased ferritin levels in the same region (Riederer et al., 1989; Jellinger et al., 1990). These discrepancies may reflect differences in data collection or disease state. The present study suggests a negative correlation between ferritin expression and LPS-induced damage to substantia nigra, providing support for the possibility that nigral dysregulation of iron metabolism is an important contributing factor to Parkinson’s disease neuropathology.

Glia expression of ferritin

Our results, in agreement with several previous studies, describe ferritin localization to microglia (Fig. 4) (Connor et al., 1994; Goto et al., 1996; Faucheux et al., 2002). In Parkinson’s disease patients, increased ferritin mRNA labeling has been observed over small glial-like cells in substantia nigra pars compacta but not in neurons (Faucheux et al., 2002). Ferritin immunoreactivity has also been demonstrated in microglial cells in substantia nigra pars compacta, substantia nigra pars reticulata, globus pallidus, striatum, and cerebral cortex of MPTP-injected monkeys, but positive staining was not observed in other glial cell types or neurons (Goto et al., 1996). While ferritin expression was not observed in astrocytes of LPS-treated rats, the possibility of ferritin expression in oligodendrocytes has not been excluded.

Alpha-synuclein accumulation in nigral dopaminergic neurons after injury

Increased levels of α-synuclein observed in dopaminergic neurons of the substantia nigra pars compacta may be related to LPS-induced iron accumulation, increased production of reactive oxygen species by activated microglia, or an interaction between iron and reactive oxygen species. In vitro, iron and oxidative stress have both been shown to promote aggregation of α-synuclein (Hashimoto et al., 1999; Ostrerova-Golts et al., 2000; Golts et al., 2002). Alpha-synuclein upregulation has also been demonstrated in mice treated with the herbicide paraquat and the neurotoxin MPTP, both of which are known to generate reactive oxygen species (Vila et al., 2000; Manning-Bog et al., 2002).

A substantial body of evidence suggests that α-synuclein accumulation may be toxic in neurons. Mutations in the α-synuclein gene are associated with familial Parkinson’s disease (Polymeropoulos et al., 1997; Kruger et al., 1998), and α-synuclein is a major component of the Lewy body, a neuropathological hallmark of Parkinson’s disease (Spillantini et al., 1997). Alpha-synuclein overexpression in vitro is associated with increased vulnerability to iron toxicity (Ostrerova-Golts et al., 2000). Further, neuronal expression of human α-synuclein in transgenic mice has been shown to cause progressive accumulation of α-synuclein and ubiquitin-immunoreactive inclusions in neurons of the substantia nigra, associated with the loss of dopaminergic terminals and motor impairments (Masliah et al., 2000; Maries et al., 2003).

In contrast to these results, however, recent studies have demonstrated a potentially neuroprotective role for α-synuclein. Transfection of α-synuclein is reported to protect a neuronal cell line from oxidative stress by inactivating c-Jun N-terminal kinase (Hashimoto et al., 2002), and α-synuclein overexpression in mice has been shown to
protect neurons of the substantia nigra pars compacta from paraquat neurotoxicity (Manning-Bog et al., 2003). While it is apparent that α-synuclein accumulation is associated with neuronal injury, the role of α-synuclein in neuronal viability remains unclear.

**Ubiquitin and proteolytic stress in Parkinson’s disease**

In the present study, accumulation of ubiquitin in dopaminergic neurons of the substantia nigra pars compacta suggests that LPS injection interferes with the ubiquitin-proteasome system, possibly through interactions between reactive oxygen species, iron, and α-synuclein. Several lines of evidence suggest that failure of the ubiquitin-proteasome system may be involved in Parkinson’s disease (Giaisson and Lee, 2003). Lewy bodies contain significant amounts of ubiquitin, and extensive accumulation of ubiquitinated proteins has been demonstrated in Lewy bodies and nigral dopaminergic neurons in postmortem tissue from Parkinson’s disease patients (Spillantini et al., 1997; McNaught et al., 2002). In addition, a rare missense mutation in the ubiquitin carboxy-terminal hydrolase L1 gene is associated with an autosomal-dominant form of familial Parkinson’s disease (Leroy et al., 1998). The ubiquitin–proteasome system protects neurons by recognizing and degrading oxidized or misfolded proteins. However, in Parkinson’s disease, factors such as increased oxidative stress, mutations in genes related to the ubiquitin–proteasome system, or proteasomal defects may lead to the buildup and compartmentalization of abnormal proteins within inclusion bodies (McNaught and Olanow, 2003). Alpha-synuclein aggregation may play a direct role in promoting proteolytic stress, as aggregated α-synuclein has been shown to inhibit ubiquitin-dependent proteasomal function in vitro (Snyder et al., 2003).

**Pallidal inflammation alters nigral physiology**

Intrapallidal injection of LPS might influence nigral ferritin, iron, α-synuclein, ubiquitin, and TH levels by several possible mechanisms. At least one study has reported direct innervation of rat SNpc by pallidal GABAergic neurons (Bevan et al., 1996), and intraneuronal transport of iron from globus pallidus to substantia nigra has been proposed to occur in Parkinson’s disease via afferent GABAergic neurons (Ryvlin et al., 1995; Double et al., 2000). However, the patterns of ferritin and iron accumulation demonstrated in Figs. 3, 4, and 5 suggest that collateral fibers projecting from SNpr to SNpc may play a more prominent role than the relatively modest nigro-pallidal connections (Prens et al., 2000). Fibers originating in globus pallidus may promote microglial activation in substantia nigra pars reticulata (Parent et al., 2000; Sato et al., 2000), leading to transmission of proinflammatory signals either by diffusion or via extensive local collaterals projecting from substantia nigra pars reticulata to substantia nigra pars compacta (Grofova et al., 1982). Given the elaborate integration of the structures of the basal ganglia, it is also possible that pallidal inflammation influences substantia nigra via neurons of the striatum or subthalamic nucleus (Blandini et al., 2000).

**CONCLUSION**

Intrapallidal injection of LPS decreases nigral TH levels while increasing iron, α-synuclein, and ubiquitin levels in the substantia nigra. The intrapallidal LPS model of Parkinson’s disease may be particularly useful in view of its differential effects with age, which have not been demonstrated with intranigral injection of LPS. Age-related deficits in ferritin induction may exacerbate LPS neurotoxicity by increasing free iron levels, leading to enhanced production of reactive oxygen species, alpha-synuclein aggregation, and ultimately dysfunction of the ubiquitin–proteasome system. Therapies aimed at reducing inflammation and normalizing cellular iron handling with age may prove beneficial in the treatment and prevention of Parkinson’s disease.

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