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Brief communication

Alpha-synuclein-glucocerebrosidase interactions in pharmacological Gaucher models: A biological link between Gaucher disease and parkinsonism

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ABSTRACT

A growing body of experimental and clinical literature indicates an association between Gaucher disease and parkinsonism, raising the possibility that convergent mechanisms may contribute to neurodegeneration in these disorders. The aim of this study was to determine whether there is a relationship between alpha-synuclein (α-syn), a key protein in Parkinson's disease pathogenesis, and abnormalities in glucocerebroside (GC) catabolism that lead to the development of Gaucher disease. We inhibited glucocerebrosidase (GCase) with conduritol B epoxide (CBE) in neuroblastoma cells and mice to test whether a biological link exists between GCase activity and α-syn. After CBE exposure, enhanced α-syn protein was detected in differentiated cells challenged with CBE as compared to vehicle, with no change in α-syn mRNA. In the mouse model, after one injection of CBE, elevated nigral α-syn levels were also detected. Analyses by Western blot and confocal microscopy revealed that normal α-syn distribution was perturbed after CBE exposure with its accumulation apparent within nigral cell bodies as well as astroglia. These findings raise the possibility that α-syn may contribute to the cascade of events that promote neuronal dysfunction in Gaucher disease and are the first to implicate this protein as a plausible biological intersection between Gaucher disease and parkinsonism using a pharmacological model.

1. Introduction

Intriguing clinical and neuropathological links have been reported between α-synucleinopathies and lysosomal storage diseases, in particular between Parkinson's disease (PD) and non-neuronopathic (type 1) Gaucher disease. Gaucher disease is the most common of the lysosomal storage diseases. It is caused by a deficiency of GCase which, under normal conditions, hydrolyzes glucocerebroside (GC) to glucose and ceramide (Butters, 2007; Choy et al., 2007; Guggenbuhl et al., 2008; Hruska et al., 2008). While over 200 different causative sequence variations in the gene that encodes for GC (*GBA*) have been reported (Jmoudiak and Futerman, 2005; Hruska et al., 2008), the majority of Gaucher disease is attributable to 11 distinct mutations resulting in one of three clinical manifestations (Pastores, 1997; Grabowski et al., 2004; Beutler, 2006; Alfonso et al., 2007). Types 2 and 3 are characterized by the presence of neurological deficits, with childhood and adolescent onset, respectively. Type 1 Gaucher disease affects both children and adults, and affects peripheral organs, but does not typically affect the nervous system. This form is most prevalent, comprising ~94% of affected individuals (Gaucher registry, https://www.lsdregistry.net/gaucherregistry/); in the Ashkenazi Jewish population, Type 1 Gaucher affects anywhere from 1 out of 500–1000 births (Grabowski, 1997; Horowitz et al., 1998; Zimran et al., 2005; Weinstein, 2007; Weinreb et al., 2008).

Clinical reports have suggested an association of type 1 Gaucher disease with a form of early onset Parkinson's disease (PD) that is often poorly responsive to levodopa (Neudorfer et al., 1996; Machaczka et al., 1999; Tayebi et al., 2001; Varkonyi et al., 2002; Bembi et al., 2003). Subsequently, a number of genetic screens of patients diagnosed with parkinsonism for sequence variants in *GBA* have also supported this association (Tayebi et al., 2003; Goker-Alpan et al., 2004; Lwin et al., 2004; Aharon-Peretz et al., 2005; Clark et al., 2005; Sato et al., 2005; Sidransky, 2006; Kono et al., 2007). For example, sequencing of the coding region for the enzyme in one such study revealed genetic variations in 14% of Parkinson's disease (PD) brain samples evaluated (Lwin et al., 2004). In another study, a Caucasian population screened for 7 *GBA* mutations was found to have sequence changes in 6% of individuals with early onset PD vs. less than 1% of controls (Sato et al., 2005). In patients of Ashkenazi-Jewish descent with parkinsonism, one or more of the six most common *GBA* mutations were identified in 31% of the population (Aharon-Peretz et al., 2005). Interestingly,

Abbreviations: GC, glucocerebroside; GCase, glucocerebrosidase; CBE, conduritol B epoxide; PD, Parkinson's disease; α-syn, alpha-synuclein; DMSO, dimethyl sulfoxide.

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increased incidence of parkinsonism is reported in relatives individuals who are obligate or confirmed carriers of a known *GBA* mutation but are asymptomatic for Gaucher disease (Goker-Alpan et al., 2004; Tayebi et al., 2003). These studies support the hypothesis that certain *GBA* mutations could be genetic risk factors for PD, and raise the possibility that even seemingly benign variations or heterozygous changes in the gene for GCase may enhance neuronal vulnerability to degenerative changes.

Further evidence for an association between PD and Gaucher disease comes from a seminal neuropathological study of Gaucher disease subjects with homozygous *GBA* mutations. This study reported four patients who were diagnosed with the type 1 Gaucher disease (with genetic confirmation), parkinsonism and dementia. In addition to classic nigral cell loss seen in PD, neuropathological findings included astrogliosis and α-syn pathology (i.e. Lewy body-like inclusions) in hippocampal CA2-4 neurons and/or the entorhinal cortex and cingulated gyrus, reminiscent of Lewy body dementia. Importantly, a correlation was revealed between the pattern of inclusion body deposition and abnormal GCase immunoreactivity. Thus, a direct relationship between neuronal GC levels and α-syn pathology may exist and/or the mechanisms involved in both processes converge and contribute to common pathophysiology.

There is little doubt regarding to the importance of α-syn in PD pathogenesis. Higher than normal expression levels of the protein have been shown to cause neurodegeneration in humans (Singleton et al., 2003; Chartier-Harlin et al., 2004; Farrer et al., 2004; Fuchs et al., 2007), and changes in α-syn levels are associated with enhanced toxicity in *in vitro* and *in vivo* PD models (Manning-Boğ et al., 2002; Vila et al., 2000; Sherer et al., 2003). Thus, depending on cellular conditions, α-syn alterations may be a risk factor for neuronal dysfunction and even frank degeneration.

Finally, a number of experimental studies support the idea that α-syn may provide a biological link between Gaucher disease and parkinsonism. Defects in GC degradation result in the accumulation of glycolipids within lysosomes, an intracellular site for protein clearance as well as lipid catabolism. Within the cell, α-syn metabolism occurs, at least in part, via the lysosomal clearance pathway (Gosavi et al., 2002; Lee et al., 2004, 2008; Ravikumar et al., 2005), and within the lysosome, α-syn binds to lipid-containing species including glycosphingolipids (Schlossmacher et al., 2005) and lipofuscin, an observation made in both PD brain (Braak et al., 2001) and mouse models of the disease (Meredith et al., 2002). Together, these reports suggest that there could be a link between GC catabolism and α-syn clearance within lysosomes. In the studies reported here, we tested the hypothesis that alterations in GCase activity affects α-syn metabolism in a way that increases intracellular levels of the protein.

2. Materials and methods

2.1. Human neuroblastoma cell line

SH-SY5Y cells were grown in Dulbecco's modified Eagle medium with 10% fetal calf serum, 2 mM glutamine, and were subcultured 1:5 with TrypLE (GIBCO/Invitrogen; Carlsbad, CA) using standard tissue culture techniques. The cells were differentiated in neurobasal media supplemented with B-27 and 40 μM retinoic acid for 7 days (Pahlman et al., 1984).

2.1.1. Conduritol B epoxide administration

Cells were exposed to CBE at doses of 0, 12.5, 25, 50, 100 or 200 μM in dimethyl sulfoxide (DMSO, Sigma Chemicals, St. Louis, MO) for 48 h at 37 °C, in duplicate, and the experiment was replicated twice. After exposure, cultures were washed with HBSS and trypsinized for 10 min followed by centrifugation at 1000 × *g* for 10 min to pellet cells. Media was removed, and cells were lysed in 10 mM Tris/1 mM EDTA/protease inhibitor cocktail (1:1000; Sigma) by sonication. Samples were centrifuged at 1000 × *g* for 10 min; the supernatant fraction was decanted from particulate fraction. Following determination of protein concentration using the BCA protein assay (Pierce Chemicals, Rockford, IL), samples were frozen until used for Western blot analysis experiments. Protein loads of 10 μg were utilized for SDS-PAGE.

2.2. Animals

Mice (C57BL/6) were maintained on a 12 h light–dark cycle (i.e. 0600–1800 lights on) and had free access to food and drinking water. All animal procedures and care methods were approved by the Institutional Animal Care and Usage Committee for the National Institutes of Health and the Parkinson's Institute.

2.2.1. Conduritol B epoxide administration

In experiments to test the effects of CBE on α-syn protein, C57BL/6 male mice, aged 8 weeks, were used in previously described paradigm with modifications (Kanfer et al., 1975, 1982; Adachi and Volk, 1977). Mice (n = 8/group) received a single i.p. injection of 200 mg/kg CBE (Sigma) in DMSO and killed 2 days after injection. To ensure changes were due to CBE and not DMSO-induced, mice injected with the vehicle were used as control. For Western blot analyses, brains were removed, dissected on ice and frozen on dry ice until needed (Manning-Boğ et al., 2002; Purisai et al., 2005). Tissues were sonicated in 10 mM Tris/1 mM EDTA/protease inhibitor cocktail (1:1000; Sigma) on ice. Samples were centrifuged at 1000 × *g* for 10 min; the supernatant fraction was decanted from particulate fraction. Following determination of protein concentration using the BCA protein assay (Pierce Chemicals, Rockford, IL), samples were frozen until used for Western blot analysis experiments. Protein loads of 10 μg were utilized for SDS-PAGE. For experiments utilizing immunohistochemistry, brains were immersion fixed in 4% paraformaldehyde and successively cryoprotected in 10 and 30% sucrose over the course of 72 h (Manning-Boğ et al., 2002, 2003). Brains were sectioned at 40-μm intervals and stored in cryopreservative solution at −20 °C until needed.

2.3. Histochemistry

Midbrain sections were immunostained using antibodies against α-syn (Syn-1, Transduction Laboratories, Lexington, KY) or glial acidic fibrillary protein (GFAP, Chemicon, Temecula, CA). Sections were then incubated with a FITC-conjugated species-specific secondary antibody and mounted onto slides as previously described (Manning-Boğ et al., 2003).

2.4. RT-PCR

RNA was extracted from human neuroblastoma cells (SH-SY5Y), treated for 24 h with CBE at varying doses or vehicle, using RAN Stat 60 (Testest, Friendswood, TX) according to manufacturer's instructions. The cDNAs were prepared by reverse transcription (Superscript III; Invitrogen). PCR was performed using the ABI PRISM 7000 Sequence Detection System and primers. The cycle number at which each PCR reaction reached a significant threshold (C_T) during the log phase of the amplification was used as a relative measure of transcript expression. The C_T of the α-syn gene was calibrated against that of the reference gene mouse HPRT.

2.5. Immunoblotting

Fractions from ventral mesencephalon separated by the centrifugation were utilized for immunoblotting experiments.

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Following homogenization in 10 mM Tris/1 mM EDTA/protease inhibitor cocktail (1:1000; Sigma, St. Louis, MO) by sonication, samples were centrifuged at 1000 × *g* for 10 min. The supernatant was decanted, and pellet fraction containing nuclei and large membrane fragments were reconstituted in homogenization buffer. The protein was measured using BCA. After proteins were separated by SDS-PAGE and transferred to nitrocellulose, the blots were blocked and incubated overnight at 4 °C with anti-α-syn (Signet; Novus Biologicals, Littleton, CO; Abcam, Cambridge, MA; Santa Cruz Biotechnology, Santa Cruz, CA) or anti-GAPDH (Sigma). Appropriate secondary antibodies conjugated to HRP were applied, and blots were incubated with a chemiluminescent substrate (Pierce) and exposed to Kodak X-Omat Blue Film (Kodak, Rochester, NY). Mouse or rabbit IgG was used in lieu of the primary antibody to ensure specificity in control experiments. Optical densities were determined using the ImageQuant program (Molecular Dynamics, Piscataway, NJ).

2.6. Statistical analyses

Differences among means were analyzed using one-way analysis of variance (ANOVA). Fisher's protected LSD post hoc analysis was employed when differences were observed in ANOVA testing ($p < 0.01$).

3. Results

3.1. In vitro effects of CBE exposure

To test the hypothesis that inhibition of GCase would elicit changes in cellular α-syn level, we evaluated the protein by using Western blot analysis in non-differentiated SH-SY5Y cells and cells differentiated to the neuronal phenotype, at 48 h following exposure to CBE. No change in α-syn was detected in non-differentiated neuroblastoma cells (data not shown); however, in differentiated SH-SY5Y cells, increased levels of α-syn protein were observed, peaking at the 50 μM dose (Fig. 1A), with a less robust effect apparent at higher CBE concentrations (Fig. 1A). In order to determine whether increased levels of the protein were due to enhanced transcription, RT-PCR was performed to measure transcript levels in SH-SY5Y treated with CBE. No change in α-syn gene expression was detected at any dose of the inhibitor tested at 24 h following CBE treatment. These findings indicate that increased α-syn levels observed following CBE exposure are not due to enhanced expression (Fig. 1B). It should be noted that no overt toxicity was noted within cells treated with GCase inhibitor (data not shown).

3.2. In vivo effects of CBE exposure

C57BL/6 mice were exposed to a single injection of CBE and assess for changes in α-syn at 48 h to determine whether diminished GCase activity is associated with alterations in the protein *in vivo*, specifically within the substantia nigra. This schedule was chosen as previous exposure paradigms have revealed enhanced α-syn levels at this timepoint (Vila et al., 2000; Manning-Boğ et al., 2002). In tissue homogenates from ventral mesencephalon of CBE vs. DMSO (vehicle)-treated mice, α-syn immunoreactivity was assessed by Western blot analysis. Denser α-syn-positive bands, representing the monomeric form of the protein (at 19 kDa), were detected in the particulate fraction at 48 h following exposure to CBE vs. DMSO, with no alteration in the supernatant fraction (Fig. 2A); densitometric analyses revealed an approximate 20% increase in ventral mesencephalon from CBE-

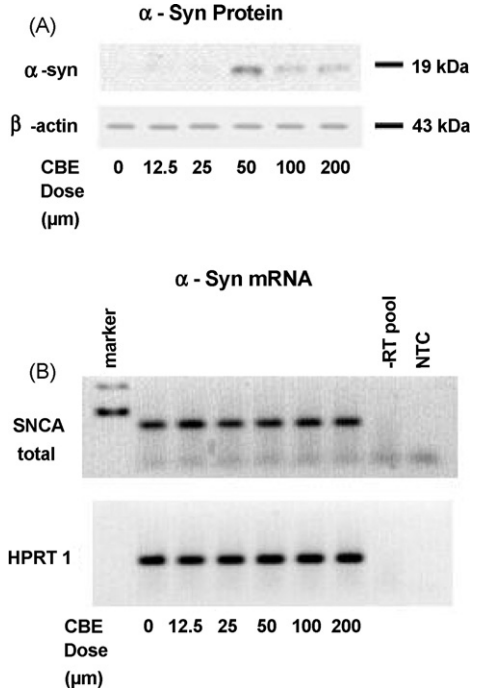

Fig. 1. α-Syn in neuroblastoma cells. (A) Differentiated SH-SY5Y cell were exposed to increasing concentrations of the GCase inhibitor CBE for 48 h. Cells were lysed and prepared for SDS-PAGE as described in Section 2. Western blot analysis showed increased levels of α-syn in cell treated with 50–200 μM CBE- vs. vehicle-treated cells. (B) Expression of α-syn mRNA was measured using RT-PCR in differentiated SH-SY5Y cells exposed to increasing concentrations of CBE for 48 h. No change in α-syn transcription was detected. Data shown are representative of experiments replicated in triplicate.

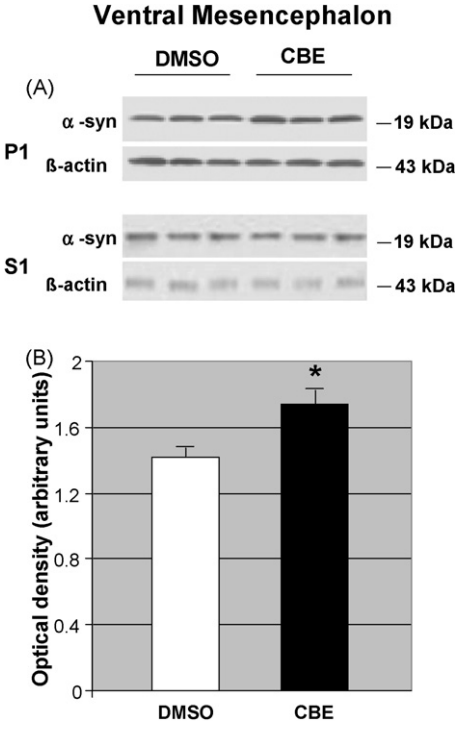

Fig. 2. α-Synuclein in ventral mesencephalon. C57BL/6 mice ($n = 8$/group) were administered a single dose of 200 mg/kg CBE or DMSO and humanely sacrificed at 48 h. (A) Representative Western blot images. Western blot of ventral mesencephalon samples showed enhanced monomeric α-syn immunoreactivity in the P1 fraction from mice treated with CBE ($n = 3$ shown) vs. DMSO-treated mice ($n = 3$ shown) with no change in the S1 fraction. (B) Densitometric analysis confirms a significant increase in α-syn levels in the P1 fraction of ventral mesencephalon from CBE- vs. DMSO-treated mice. $^*p < 0.01$.

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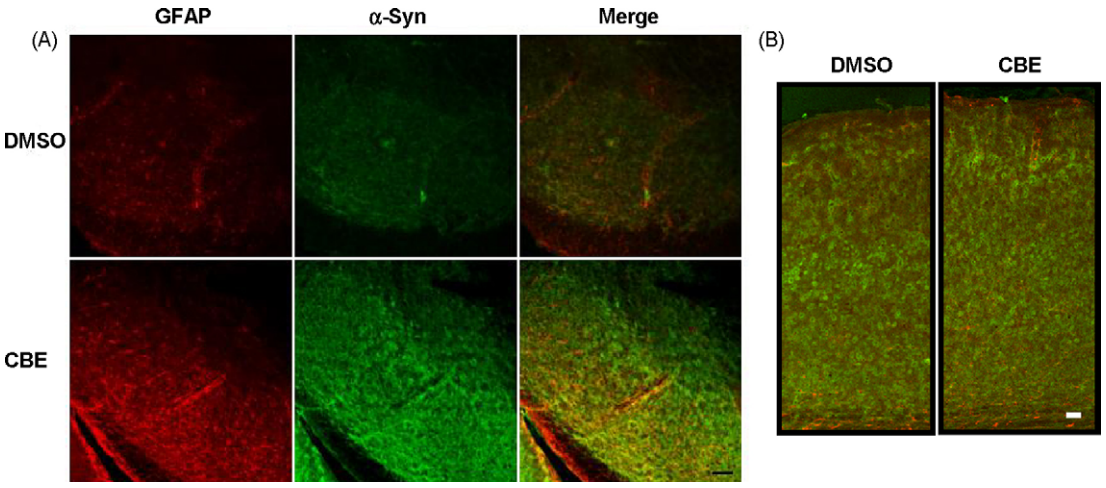

Fig. 3. α-Synuclein in mouse brain. C57BL/6 mice (n = 8/group) were administered a single dose of 200 mg/kg CBE or DMSO and humanely sacrificed at 48 h. Dual-label immunohistochemistry was performed in coronal brain sections from substantia nigra (A) and cortex (B) to detect α-syn (FITC secondary) and GFAP (Cy3 secondary). Representative images are shown. (A) Immunohistochemical experiments revealed an apparent increase in the levels of GFAP and α-syn in the substantia nigra of CBE- vs. vehicle-treated mice. Bar = 50 μm. (B) GFAP and α-syn immunoreactivity in cortex from CBE- and vehicle-treated mice. The merged images demonstrate no apparent difference for either protein in the cortex. Bar = 20 μm.

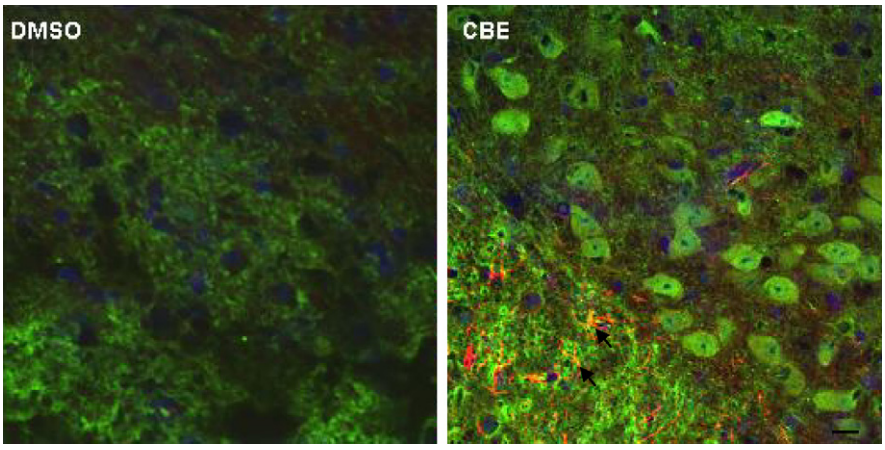

Fig. 4. Neuronal and glia α-synuclein the substantia nigra. Dual-label immunostaining for α-syn (FITC secondary) and GFAP (Cy3 secondary) was performed using sections from the substantia nigra from DMSO and CBE-treated mice. Hoescht staining (DAPI) was used to identify nuclei. Representative images are shown. Analyses at higher magnification clearly demonstrate a dramatic accumulation of α-syn within cell bodies and processes in the substantia nigra of CBE- vs. vehicle-treated mice. Glial activation was particularly robust within neuropil of CBE-treated mice, as shown by GFAP immunohistochemistry. Co-localization of α-syn and GFAP immunoreactivities were apparent in the merged image, and indicate positive α-syn staining within astrocytes of CBE-treated mice (arrows). Bar = 20 μm.

treated animals as compared to control (Fig. 2B). No immunoreactivity for higher molecular forms of α-syn (i.e. SDS-stable aggregates) was observed under these conditions (data not shown).

The effects of CBE exposure on α-syn within the ventral mesencephalon were also assessed histologically with immunohistochemistry. Coronal sections containing substantia nigra from mice at 48 h after CBE or DMSO exposure were immunostained using an antibody derived against α-syn (i.e. Syn-1). Subsequent evaluation of the sections revealed that robust immunoreactivity was observed within the cell bodies of the substantia nigra pars compacta of treated vs. control mice (Figs. 3A and 4), and enhanced immunoreactivity for α-syn was detected within the cytoplasm and cell nuclei (Figs. 3A and 4) of A9 neurons, reminiscent of the α-syn response in PD models of toxicant exposure (Vila et al., 2000; Manning-Boğ et al., 2002; Goers et al., 2003). No obvious changes in α-syn were observed in other brain regions, such as the cortex (Fig. 3B) and hippocampus (data not shown), 48 h following a single administration of CBE to mice.

Substantia nigra-containing tissue sections were immunostained using an antibody for the astrocytic marker, glial fibrillary acidic protein (GFAP). At 48 h after exposure to a single systemic treatment, astroglial activation, as observed by GFAP immunoreactivity, was apparent in the substantia nigra (Fig. 4). Interestingly, dual-label immunofluorescence analysis revealed that enhanced α-syn was also detected within activated astrocytes of the substantia nigra following CBE exposure (Fig. 4), suggesting that similar mechanisms (e.g., abnormal protein accumulation and/or trafficking) may be at play in astroglia as well as neurons in the model.

4. Discussion

A variety of studies have suggested the possibility of an association between Type 1 Gaucher disease and PD. In this report we provide experimental evidence for a potential biological link between the disorders by demonstrating two key changes in cellular and mouse models after pharmacological GCase inhibition

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with CBE, the enzyme that is defective in Gaucher disease. In tissue culture, we observed increased immunoreactivity for α-syn within CBE-exposed neuroblastoma cells differentiated to the neuronal phenotype. In mice we observed increased α-syn immunoreactivity within the substantia nigra, the key nucleus that is affected in PD, after a single treatment of GCase inhibitor. Interestingly, CBE administration also induced astrocytic activation within the substantia nigra of exposed mice, reminiscent of the astrogliosis observed in human Gaucher brain (Cervos-Navarro and Zimmer, 1990) and post-mortem tissue obtained from patients with Gaucher disease and manifested parkinsonism during life (Wong et al., 2004). These data suggest that neurodegenerative events may be promoted in the *in vivo* CBE model. Furthermore, these observations show that reduced GCase activity promotes alterations in α-syn in the substantia nigra. Given the prominent role that this protein plays in PD, these results could be providing a clue to a mechanism that underlies the predisposition of dopaminergic neurons to damage in Gaucher disease associated parkinsonism.

Treatment of neuroblastoma cells resulted in enhanced monomeric α-syn protein immunoreactivity by Western blot (Fig. 1A); interestingly, the peak increase was detected using 50 μM with a less robust effect at the 100 and 200 μM doses. Although this observation could be explained by cell injury and death with the higher CBE concentrations, no apparent toxicity was noted at any concentration used in the experiments (data not shown). Alternatively, it is possible that the less robust effect on monomeric α-syn levels at the higher doses may be due to oligomerization of α-syn (Kalivendi et al., 2004), but the higher molecular weight forms are below the detection limit of the assay.

The alteration in α-syn after CBE administration noted in this report is reminiscent of observations in other toxicant-exposure models of parkinsonism. Indeed, a similar increase in α-syn levels in the substantia nigra have been observed in mice exposed to the herbicide paraquat (Manning-Boğ et al., 2002), the pesticides rotenone (Sherer et al., 2003; Betarbet et al., 2006) and dieldrin (Hatcher et al., 2007) and the parkinsonism-producing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Vila et al., 2000); all of these models elicit nigrostriatal injury and/or frank dopaminergic cell death (Burns et al., 1983; Heikkila et al., 1984; Langston et al., 1984a,b; Betarbet et al., 2000; McCormack et al., 2002; Hatcher et al., 2007). Given that increased α-syn levels in patients with genomic duplications and triplications of the SNCA gene lead to autosomal dominant parkinsonism, with α-syn pathology and nigral cell loss (Singleton et al., 2003; Chartier-Harlin et al., 2004; Farrer et al., 2004; Fuchs et al., 2007), it is possible that enhanced protein concentrations may promote toxicity within the CBE model as well.

Surprisingly, enhanced α-syn immunoreactivity was also apparent within astroglia of CBE-treated mice, showing that GCase inhibition increases levels of the protein within astrocytes as well as nigral neurons. To the best of our knowledge, this is the first demonstration of α-syn alterations within astrocytes in an *in vivo* model of any disease. Whether enhanced α-syn within astrocytes represents a primary event (i.e. due to astroglial GCase inhibition) or a secondary response to neuronal injury is not clear, but its presence under these conditions could be relevant to both Gaucher disease and PD. While oligodendrocytes contain α-syn-positive depositions, known as glial cytoplasmic inclusions, in another form of parkinsonism (i.e. multiple system atrophy), less attention has been paid to astroglia in parkinsonian disorders. However, two different laboratories have observed α-syn-immunoreactive astrocytes in sporadic PD brain. Braak et al. (2007) and one of these groups have suggested that such changes occur quite early in the evolution of the disease. Our unexpected results may provide the first *in vivo* model to study this phenomenon.

While the mechanism for increased levels of the protein in astrocytes in PD is not readily apparent, it is possible that extracellular α-syn released from neurons is taken up into surrounding astroglia; indeed, such events have been hypothesized to contribute to astrocytic activation (Croisier and Graeber, 2006; Braak et al., 2007; Lee, 2008). Alternatively, it may be that upregulation is responsible for increased α-syn levels in astrocytes. α-Syn has been detected in cultured human astrocytes, and its expression level is responsive to cytokine exposure (Tanji et al., 2001). Recently, Vitner and Futerman found that α-syn is transcriptionally regulated in astroglial cultures challenged with CBE (2008). Our investigation extends these observations to an *in vivo* model of the phenomenon that could be used to study PD and possibility Gaucher disease. In this regard, it would be of interest to examine α-syn in astrocytes in all types of Gaucher disease, including those associated with PD.

However, it is important to additional factors, rather than upregulation, contribute to increased α-syn levels in neurons; indeed, our experiments in neuroblastoma cells demonstrate that α-syn transcript is not altered 24 h after CBE treatment (Fig. 1). A possible alternative is decreased lysosomal α-syn clearance and/or binding of the protein to accumulating glycolipids (Lee et al., 2004; Schlossmacher et al., 2005). Such changes could lead to an alteration of normal α-syn metabolism, trafficking and ultimately, function. In support of this possibility, our studies show that the normal cellular distribution of α-syn is perturbed after GCase inhibition by CBE. After CBE exposure to mice, we found accumulation of the protein within neuronal cell bodies (Figs. 3 and 4), and further, that ventral mesencephalon levels of α-syn were increased in the particulate fraction (Fig. 2), suggesting an alteration in α-syn solubility and/or its trafficking. Under normal conditions, α-syn co-localizes with lipid rafts that mediate its delivery to the synapse, but under conditions of altered lipid metabolism, this association is disrupted (Fortin et al., 2004). Consequently, redistribution of the protein to the cell body from neurites occurs, a scenario that could lead to the formation of abnormal and potentially toxic α-syn species (Fortin et al., 2004). In this setting, disruption of normal α-syn–lipid interactions, due to diminished GCase activity or other regulators of lipid metabolism, could well represent a pathway that leads to cellular demise. Whether diminished GC degradation promotes α-syn upregulation, decreased protein degradation and/or abnormal intracellular α-syn distribution/trafficking, our studies indicate that: (i) the CBE paradigm shows promise as a tool for evaluating the role of α-syn–lipid interactions in neuronal and potentially glial vulnerability and (ii) these pathways represent a plausible biological link between Gaucher disease and related parkinsonism.

Conflict of interest

The authors of this study declare that there are no conflicts of interest.

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