The Convergence of Dopamine and α-Synuclein: Implications for Parkinson's Disease

Danielle E Mor[1] and Harry Ischiropoulos[2]

[1]Lewis-Sigler Institute for Integrative Genomics and Department of Molecular Biology, Princeton University, Princeton, NJ, USA. [2]Perelman School of Medicine, University of Pennsylvania, and the Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA.

ABSTRACT: In Parkinson's disease (PD), the loss of dopamine-producing neurons in the substantia nigra (SN) leads to severe motor impairment, and pathological inclusions known as Lewy bodies contain aggregated α-synuclein protein. The relationship of α-synuclein aggregation and dopaminergic degeneration is unclear. This commentary highlights a recent study showing that the interaction of α-synuclein with dopamine may be an important mechanism underlying disease. Elevating dopamine levels in mice expressing human α-synuclein with the A53T familial PD mutation recapitulated key features of PD, including progressive neurodegeneration of the SN and decreased ambulation. The toxicity of dopamine was dependent on α-synuclein expression; hence, raising dopamine levels in nontransgenic mice did not result in neuronal injury. This interaction is likely mediated through soluble α-synuclein oligomers, which had modified conformations and were more abundant as a result of dopamine elevation in the mouse brain. Specific mutation of the dopamine interaction motif in the C-terminus of α-synuclein rescued dopamine neurons from degeneration in *Caenorhabditis elegans* models. Here, these findings are discussed, particularly regarding possible mechanisms of oligomer toxicity, relevance of these models to sporadic and autosomal recessive forms of PD, and implications for current PD treatment.

KEYWORDS: Neurodegeneration, Parkinson's, dopamine, α-synuclein, oligomer

Parkinson's disease (PD) is the second most common neurodegenerative disorder behind Alzheimer's disease, affecting more than 7 million people worldwide and predicted to increase significantly as the population ages.[1,2] PD is primarily but not exclusively a motor disorder, characterized by resting tremor, muscle rigidity, slow movement, and postural instability. These symptoms are largely due to the progressive loss of dopamine-producing neurons in the substantia nigra (SN). Neurons in the SN and other brain regions also develop abnormal intracellular protein-rich deposits known as Lewy bodies.[3] Shortly after the discovery of a mutation in the gene encoding the presynaptic protein α-synuclein in families with dominantly inherited PD,[4] it was found that Lewy bodies contain aggregated α-synuclein even in the sporadic form of the disease.[5] The relationship of these 2 defining features of PD—degeneration of dopamine neurons and α-synuclein aggregation—is unknown; however, our recent study[6] offers a possible mechanism that may bridge these characteristic pathologies.

Despite the clear link between α-synuclein and disease, it has been difficult to produce dopaminergic cell death in mice by the transgenic expression of either wild-type or familial PD mutants of α-synuclein. A possible explanation for this is that critical disease factors are not at play in these models, and one such factor may be dopamine itself. Dopamine oxidation, which occurs spontaneously in the cytosol as well as enzymatically during metabolism, generates electrophilic quinones and reactive oxygen species.[7,8] These oxidation products are known to be cytotoxic in vitro,[9] yet few studies have examined whether dopamine can compromise neuronal health in vivo, and may thereby play a causative role in PD.

To investigate the toxicity of dopamine and its possible synergism with α-synuclein in vivo, we manipulated levels of both dopamine and α-synuclein in aged (10-month-old) mice.[6] We achieved long-term elevation of dopamine using a lentiviral vector targeted to the SN. This vector carried the gene for tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis, mutated at 2 residues (R37E and R38E) that are critical for feedback inhibition by dopamine (TH-RREE).[10–12] We first tested the effects of increasing striatal dopamine levels (by more than 50%) in nontransgenic mice, and surprisingly we found that 5 months postinjection (mpi), there was no evidence of neurotoxicity. Although we did not directly test for markers of oxidative stress, we found that there was no loss of dopaminergic neurons or synapses.[6] Moreover, the mice were hyperactive, which is consistent with excess dopamine being packaged into vesicles (where it remains reduced) and released into the synapse.[13] Although the exact mechanisms by which cells were able to tolerate an overabundance of dopamine are not known, upregulation of the dopamine transporter (DAT) in

these mice suggests that dynamic tuning of dopamine handling/metabolic systems is sufficient to protect against dysregulated dopamine levels.

Strikingly, when we increased dopamine levels in mice expressing human α-synuclein with the A53T familial PD mutation,[14] we observed a very different response. Within 2.5 months after TH-RREE vector injection, dopaminergic synapses in the striatum were reduced by 25%, whereas cell bodies in the SN remained intact, as compared with control vector–injected A53T mice. By 5 mpi, striatal denervation had progressed to 62% loss of dopaminergic contacts, and there was a 25% decrease in the number of cell bodies in the SN.[6] The loss of nerve terminals prior to the disappearance of cell bodies mimics the disease progression in patients with PD, in which there is evidence for degeneration beginning at the synapse, causing a "dying back" of the axon and eventually death of the soma.[15] It appears that the early loss of synapses in A53T TH-RREE mice already translated into a functional deficit because these mice were not hyperactive despite elevated dopamine levels at this time point (2.5 mpi). Striatal dopamine levels dropped by 37% from 2.5 to 5 mpi, presumably the result of ongoing neurodegeneration and the mice became hypoactive. Interestingly, A53T TH-RREE mice had reduced ambulation at 5 mpi despite the fact that their dopamine levels had not fallen below those of controls.[6] This may reflect a functional imbalance among synapses that had degenerated and those that remained and were still overproducing dopamine (from TH-RREE expression).

This new mouse model, which undergoes nigrostriatal degeneration and an associated locomotor impairment, indicates that the combination of dyregulated dopamine and α-synuclein can have dire consequences for the cell. The nature of this interaction may be direct, as in the noncovalent reaction of oxidized dopamine with α-synuclein which has been observed in vitro,[10,11,16,17] or indirect, for example, through dysfunction of dopamine regulatory systems as a result of α-synuclein expression. In the latter case, A53T mice may have an impairment in compensatory mechanisms that were able to successfully maintain dopamine homeostasis in nontransgenic TH-RREE mice. It is not known whether DAT levels were upregulated in response to excess dopamine in A53T TH-RREE mice, since the substantial loss of synapses the substantial loss of synapses caused an overall decrease in DAT.[6] However, we previously found that A53T mice naturally have higher dopamine levels than nontransgenic mice,[18] and in this study, we documented this as well as higher basal DAT levels,[6] suggesting that dopamine regulatory mechanisms are intact. Future investigations may wish to use this model to examine the effects of α-synuclein on other components of dopamine regulation and metabolism, particularly in the context of the stress of excess dopamine.

To test the possibility of a more direct mechanism of toxicity, we attempted to rescue neurons from degeneration by disrupting the noncovalent interaction between dopamine and α-synuclein. Using the genetically tractable model organism, *Caenorhabditis elegans*, we first expressed human A53T α-synuclein in dopamine neurons and observed neurodegeneration, consistent with prior reports.[19,20] Crossing these animals with those overexpressing CAT-2, the worm homologue of tyrosine hydroxylase, significantly enhanced neurotoxicity.[6] Next, we expressed CAT-2 along with A53T α-synuclein that was specifically mutated at the site of interaction with dopamine (the $Y_{125}EMPS_{129}$ motif in the C-terminus).[11,17] Remarkably, these worms were completely resistant to the neurotoxicity of CAT-2, suggesting that dopamine induces neurodegeneration in vivo through its reaction with the C-terminus of α-synuclein.

Intriguingly, the incubation of dopamine with α-synuclein in vitro results in the stabilization of potentially toxic α-synuclein oligomers, and the $Y_{125}EMPS_{129}$ motif in the C-terminus is required for this effect.[10,11,16,17] α-Synuclein oligomers, which are intermediate species generated during the aggregation process, are increasingly suspected of playing a causative role in disease.[21] To investigate for the first time in vivo the effects of dopamine on α-synuclein oligomerization, soluble α-synuclein was extracted from the SN of A53T TH-RREE mice and A53T controls at 5 mpi. Characterization of α-synuclein species using native size-exclusion chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that dopamine increased total steady-state levels of oligomers and promoted larger oligomer conformations.[6] Dopamine-induced neurodegeneration may therefore be mediated by soluble α-synuclein oligomers, providing a link between dopamine toxicity and α-synuclein aggregation.

These findings raise many important questions, particularly regarding the mechanisms by which dopamine-induced α-synuclein oligomers may drive disease. Several potential mechanisms have been proposed, including membrane damage, mitochondrial dysfunction, endoplasmic reticulum stress, neuroinflammation, and impairment of protein degradation systems.[22] In vitro, dopamine-stabilized α-synuclein oligomers inhibit SNARE complex assembly, resulting in reduced neurotransmitter release.[23] It is therefore possible that the decline in striatal dopamine levels from 2.5 to 5 mpi in A53T TH-RREE mice[6] was the result of a defect in dopamine release. In this case, synaptic dysfunction may have led first to degeneration of the nerve terminal, followed eventually by loss of the cell body, which is consistent with the disease progression we observed. Dopamine-stabilized oligomers also impair chaperone-mediated autophagy in vitro,[24] and this mechanism may contribute to cell death in vivo. Further investigation is necessary to determine whether these and other cellular processes are responsible for neurodegeneration in A53T TH-RREE mice and the *C elegans* models.

Another critical question is the relevance of the A53T α-synuclein mouse and worm models to sporadic PD and to other familial forms of the disease. It must first be said that both sporadic PD and PD caused by the A53T mutation in α-synuclein are characterized by loss of dopaminergic neurons and abundant Lewy body pathology. In sporadic cases, Lewy bodies contain aggregates of wild-type α-synuclein.[3] The A53T mouse model develops widespread α-synuclein aggregation,[14] and with enhancement of dopamine levels, undergoes progressive degeneration of the SN, an eventual depletion of striatal dopamine, and hypokinesia similar to the human condition.[6] In our study and others, the expression of either A53T or wild-type α-synuclein in *C elegans* induces dopaminergic degeneration.[6,19,20] Moreover, the interaction of dopamine and α-synuclein is not specific to the A53T-mutant protein. We showed using recombinant wild-type human α-synuclein incubated with dopamine in vitro that the resulting oligomer species share biochemical properties with oligomers derived from A53T TH-RREE mice and reduce cell viability in primary neuronal cultures.[6] To further establish relevance to sporadic PD, dopamine levels can be elevated in mice overexpressing wild-type human α-synuclein.

Autosomal recessive forms of PD, caused by mutations in the *parkin, PINK1*, and *DJ1* genes, are similar to sporadic PD in terms of motor symptoms and SN degeneration but typically lack Lewy bodies.[3] Although the role of α-synuclein in these cases is unclear, the A53T TH-RREE and *C elegans* models raise the possibility that α-synuclein oligomers, and, in particular, dopamine-induced species, may be as yet unappreciated sources of toxicity in these forms of the disease. In addition, defects in mitochondrial autophagy have been implicated in autosomal recessive PD.[3] It would be informative to use the new mouse and worm models to examine the relationship between mitochondria, α-synuclein, and dopamine and thereby potentially better understand the shared and distinct mechanisms among different types of PD.

Finally, a potential implication of our work is that clinical use of the dopamine precursor, L-DOPA (L-3,4-dihydroxyphenylalanine), as treatment for PD may hasten neurodegeneration. Although more studies are needed, there is currently no conclusive evidence from patients with PD indicating that L-DOPA accelerates the disease.[25] Moreover, by the time motor symptoms have manifested and patients are seeking treatment, a substantial percentage of SN cells are already lost.[15] The L-DOPA therapy provides significant symptomatic relief and therefore we advocate a cautious approach to the interpretation of our findings regarding this issue.

Taken together, the data from our recent study[6] suggest the following scenario for neuronal demise in PD. Neurons may form α-synuclein oligomers as a result of stochastic processes, and if these species are able to escape proper clearance due to age-related decline in protein quality control, they may come in contact with cytosolic dopamine and become stabilized and/or modified. The resulting species may then participate in aberrant interactions in the cell, ultimately leading to neurodegeneration.

Targeting dopamine-induced α-synuclein oligomers may offer new hope in the search for disease-modifying therapies for PD.

Author Contributions

DEM wrote the commentary and HI provided important edits.

REFERENCES

1. Van Den Eeden SK, Tanner CM, Bernstein AL, et al. Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. *Am J Epidemiol*. 2003;157:1015–1022.
2. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol*. 2006;5:525–535.
3. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. *Nat Rev Neurol*. 2013;9:13–24.
4. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 1997;276:2045–2047.
5. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in lewy bodies. *Nature*. 1997;388:839–840.
6. Mor DE, Tsika E, Mazzulli JR, et al. Dopamine induces soluble α-synuclein oligomers and nigrostriatal degeneration. *Nat Neurosci*. 2017;20:1560–1568.
7. Graham DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol*. 1978;14:633–643.
8. Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology*. 1996;47:S161–S170.
9. Sulzer D, Zecca L. Intraneuronal dopamine-quinone synthesis: a review. *Neurotox Res*. 2000;1:181–195.
10. Mazzulli JR, Mishizen AJ, Giasson BI, et al. Cytosolic catechols inhibit alpha-synuclein aggregation and facilitate the formation of intracellular soluble oligomeric intermediates. *J Neurosci*. 2006;26:10068–10078.
11. Mazzulli JR, Armakola M, Dumoulin M, Parastatidis I, Ischiropoulos H. Cellular oligomerization of alpha-synuclein is determined by the interaction of oxidized catechols with a C-terminal sequence. *J Biol Chem*. 2007;282:31621–31630.
12. Nakashima A, Kaneko YS, Mori K, et al. The mutation of two amino acid residues in the N-terminus of tyrosine hydroxylase (TH) dramatically enhances the catalytic activity in neuroendocrine AtT-20 cells. *J Neurochem*. 2002;82:202–206.
13. Lohr KM, Bernstein AI, Stout KA, et al. Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. *Proc Natl Acad Sci USA*. 2014;111:9977–9982.
14. Giasson BI, Duda JE, Quinn SM, Zhang B, Trojanowski JQ, Lee VM. Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. *Neuron*. 2002;34:521–533.
15. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol*. 2010;67:715–725.
16. Conway KA, Rochet JC, Bieganski RM, Lansbury PT Jr. Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science*. 2001;294:1346–1349.
17. Norris EH, Giasson BI, Hodara R, et al. Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations. *J Biol Chem*. 2005;280:21212–21219.
18. Tsika E, Moysidou M, Guo J, et al. Distinct region-specific alpha-synuclein oligomers in A53T transgenic mice: implications for neurodegeneration. *J Neurosci*. 2010;30:3409–3418.
19. Lakso M, Vartiainen S, Moilanen AM, et al. Dopaminergic neuronal loss and motor deficits in *Caenorhabditis elegans* overexpressing human alpha-synuclein. *J Neurochem*. 2003;86:165–172.
20. Cao S, Gelwix CC, Caldwell KA, Caldwell GA. Torsin-mediated protection from cellular stress in the dopaminergic neurons of *Caenorhabditis elegans*. *J Neurosci*. 2005;25:3801–3812.
21. Kayed R, Head E, Thompson JL, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science*. 2003;300:486–489.
22. Roberts HL, Brown DR. Seeking a mechanism for the toxicity of oligomeric α-synuclein. *Biomolecules*. 2015;5:282–305.
23. Choi BK, Choi MG, Kim JY, et al. Large alpha-synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. *Proc Natl Acad Sci USA*. 2013;110:4087–4092.
24. Martinez-Vicente M, Talloczy Z, Kaushik S, et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. *J Clin Invest*. 2008;118:777–788.
25. Olanow CW. Levodopa: effect on cell death and the natural history of Parkinson's disease. *Mov Disord*. 2015;30:37–44.