α-Synuclein Oligomerization by Dihydroxyphenylacetaldehyde (DOPAL)

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ABSTRACT

Background: Parkinson disease (PD) is characterized by abnormal accumulations of α-synuclein in dopaminergic neurons (Lewy bodies). Mechanisms of α-synuclein aggregation in dopaminergic neurons remain obscure. This project focused on effects of the catecholaldehyde metabolite of dopamine (DA), dihydroxyphenylacetaldehyde (DOPAL) on α-synuclein.

Methods: Western blots were obtained after incubation of α-synuclein with DA, DOPAL, and dihydroxyphenylacetic acid (DOPAC). Wild-type α-synuclein as well as the A53T and A30P mutated forms were studied.

Results: DOPAL oligomerized α-synuclein remarkably. DA was less effective and DOPAC ineffective. Mutated α-synuclein was associated with presumed aggregation by DOPAL, such that the protein did not migrate in the gel.

Discussion: This study demonstrates that DOPAL potently oligomerizes α-synuclein and appears to aggregate mutant forms of the protein. These effects may provide clues to the formation of Lewy bodies in dopaminergic neurons in sporadic and familial PD.

INTRODUCTION

Parkinson Disease (PD) is one of the most prevalent neurodegenerative diseases in the elderly. PD is characterized by a movement disorder thought to result from loss of dopaminergic neurons in the substantia nigra (SN) that project to the basal ganglia. Loss of dopaminergic terminals in the distal putamen is especially prominent (Figure 1).

A pathologic hallmark of PD is Lewy bodies, abnormal proteinaceous material in SN neurons. Lewy Bodies contain aggregates of protein, α-synuclein. The functions of α-synuclein remain obscure, but mutation of the gene encoding this protein was the first identified genotypic change found to cause familial PD. α-Synuclein is expressed ubiquitously, and reasons for α-synuclein precipitation in dopaminergic neurons remain obscure.

This project explored one explanation for the relationship between α-synuclein and dopamine—the "catecholaldehyde hypothesis." According to this hypothesis, catecholamines such as dopamine that are present in the neuronal cytoplasm are metabolized by monoamine oxidase (MAO) to form catecholaldehydes, which are toxic. The catecholaldehyde formed from oxidative deamination of dopamine is dihydroxyphenylacetaldehyde, or DOPAL. DOPAL is metabolized by aldehyde dehydrogenase (AD) to dihydroxyphenylacetic acid (DOPAC), an acidic metabolite that exits the neuron.

The objective of this study was to determine whether and to what relative extents DA, DOPAL, and DOPAC result in oligomerization of α-synuclein. We examined effects of these three catechols on three types of α-synuclein—wild type, A53T, which was the first mutation found to underlie familial PD (1), and A30P.

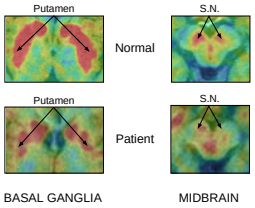

Figure 1: *Positron emission tomographic (PET) scans after injection of 6-[^{18}F]fluorodopa, showing decreased 6-[^{18}F]fluorodopa-derived radioactivity in the substantia nigra (SN) and putamen in a patient with Parkinson disease.*

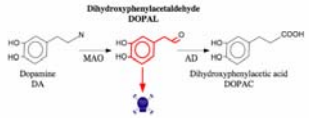

Figure 2: *The catecholaldehyde hypothesis. According to this hypothesis, DOPAL, an intermediate metabolite of dopamine, is neurotoxic.*

METHODS/PROCEDURES

Reagents

Stock solutions of dopamine (DA) HCl (1 mg/mL) and diydroxyphenylacetic acid (DOPAC) Sigma Aldrich, St. Louis, MO) in in Type-1 water were diluted serially to 1.0μM, 0.10μM, and 0.01μM. Dihydroxyphenylacetaldehyde (DOPAL) was synthesized in the laboratory of Dr. Kenneth Kirk (NIDDK) and α-synuclein in the laboratory of Dr. Nelson Cole (NINDS/NHLBI).

Incubations

Solutions of α-synuclein (5 μg in 100 ul PBS) were mixed with 0.01 μM, 0.1 μM, or 1.0 μM of DA, DOPAC, or DOPAL in 1.5mL sealed sample tubes (Sarstedt, Germany) and incubated overnight at 37˚C. After overnight incubation, samples (5 μl) were transferred into fresh tubes and loaded with loading buffer (Nu Page LDS Sample Buffer; Invitrogen), Magic Mark XP Western Standard (Invitrogen), See Blue Plus 2 Prestained Standard, and Nu Page Sample Reducing Agent (Invitrogen). Samples were then heated at 70˚C for 10 min and loaded onto 4-12% Bis-Tris gels (Invitrogen). The total amount of protein loaded on the gel was adjusted to 20 μg per lane. Reference markers with known molecular weights (Magic Marker XP and SeeBlue Plus 2, Invitrogen) were run in the same gels (XCell SureLockTM Mini-Cell, Invitrogen) at constant 200 V for 60 min using MOPS SDS running buffer.

Protein Transfers and Western Blots

The proteins were transferred from gel to Immobilon-P polyvinylidene fluoride (PVDF) membranes (Millipore) at 85 V (150 mA) for 1.5 hrs using XCell II blotting apparatus (Invitrogen). The membrane was allowed to dry overnight. The membrane was blocked using a blocking buffer (TBS with 0.1% Tween 20 and 5% non-fatty dried milk) for 1 hr and then incubated in the blocking buffer with IgG2a mouse monoclonal [Syn 202] primary antibody (1:1000 dilution; Abcam, Cambridge, MA) on a shaker at room temperature for 2 hrs. Upon completion of primary incubation, the membrane was washed 3 times in TBS/Tween20 for 5 min, then incubated for 1 hr on a shaker with a secondary antibody (Rabbit polyclonal to Mouse IgG-H&L [HRP] Abcam, Cambridge, MA) at a dilution of 1:20,000 in blocking buffer, and then washed 4 times (5-min/wash). After four washing cycles the membranes were immersed in chemiluminescent substrate (Western Blot Detection Reagent (Amersham Biosciences, Little Chalfont, UK), exposed to Hyperfilm ECL film (Amersham Biosciences, Little Chalfont, UK) and developed.

RESULTS

About 10-fold greater oligomerization of α-synuclein was observed upon incubation with DOPAL than with DA, and about 10-fold greater oligomerization with DOPAL than with DOPAC (Figure 3).

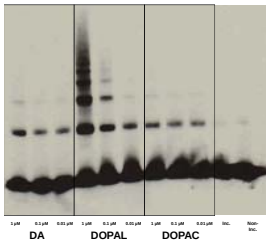

Figure 3: *Oligomerization of wild-type α-synuclein by various concentrations of DA, DOPAL, and DOPAC. DOPAL potently oligomerized α-synuclein.*

Oligomerization of α-synuclein was also prominent upon incubation of DOPAL with the A53T and A30P variants (Figures 4 and 5). A substantial amount of protein did not migrate, upon incubation of DOPAL with A53T (box in Figure 4).

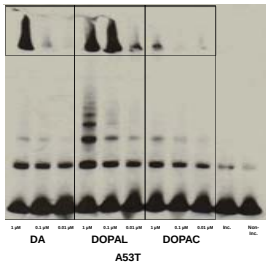

Figure 4: *Oligomerization of the A53T variant of α-synuclein by various concentrations of DA, DOPAL, and DOPAC. DOPAL potently oligomerized α-synuclein. Box indicates non-migration of α-synuclein in the gel.*

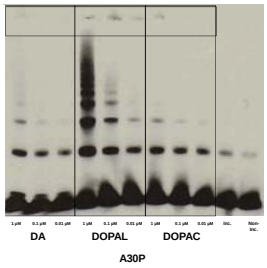

Figure 5: *Oligomerization of the A30P variant of α-synuclein by various concentrations of DA, DOPAL, and DOPAC. DOPAL potently oligomerized α-synuclein. Box indicates non-migration of α-synuclein in the gel.*

DISCUSSION

The main new findings from these experiments are that the catecholaldehyde, DOPAL, potently oligomerizes α-synuclein and that incubation of DOPAL with the A53T mutant form of the protein results in a substantial amount of non-migration of α-synuclein in the gel. These findings provide clues about possible relationships between α-synuclein and catecholamine metabolism. Individuals with augmented delivery of DA to the neuronal cytoplasm, such as by decreased activity of the vesicular monoamine transporter, or with decreased ability to metabolize DOPAL by aldehyde dehydrogenase, might be susceptible to oligomerization of α-synuclein because of the buildup of DOPAL in the cytoplasm. If failure to migrate in the gel reflected aggregation of the protein, then individuals carrying the A53T mutation might be especially susceptible to damaging effects of DOPAL.

Our findings support previous studies showing that DA can oligomerize α-synuclein (2); however, DOPAL is about 10 times as potent in this regard. The acidic metabolite of DOPAL, DOPAC, is less potent than DA.

Planned follow-up studies will extend to cellular models. In rat pheochromocytoma cells (PC-12 cells), manipulations that increase endogenous DOPAL production augment cytotoxic effects of the metabolic stressor, rotenone (3), and exposure to exogenous DOPAL evokes precipitation of α-synuclein (2). PC-12 cells expressing A53T mutant α-synuclein would be expected to be especially susceptible to catecholaldehyde-induced α-synuclein precipitation and apoptotic cell death.

REFERENCES

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CONCLUSIONS

--The catecholaldehyde, DOPAL, potently oligomerizes α-synuclein.
--DOPAL effects on the A53T mutant form of α-synuclein include non-migration of the protein.