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 A30T and A35P 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.

**CONCLUSIONS**

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

REFERENCES


**METHODS/PROCEDURES**

**Reagents**

Stock solutions of dopamine (DA) HCl (1 mg), and dihydroxyphenylacetic acid (DOPAC) Sigma Aldrich, St. Louis, MO. All 6 µL volumes were diluted serially to 1.0 µM, 0.10 µM, and 0.01 µM. 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 µL of PBS) were mixed with 0.0, 0.1, 0.01, and 0.1 µM of DA, DOPAL, or DOPAC in 1.0 ml sealed sample tubes (Sarstedt, Germany) and incubated overnight at 37 °C. After overnight incubation, samples (1 µl) were transferred into fresh tubes and loaded using loading buffer (20 µM LDS sample buffer, Invitrogen, White, Rockford, IL) and loaded on a precast gel (Invitrogen). The gel was run at 85 V (150 mA) for 1 hr, 150 V (225 mA) for 0.5 hr and 250 V (350 mA) for 0.5 hr. The gels were stained with Coomassie blue R-250 for 20 min at room temperature. The membranes were run in the same gel (NC-SCG/NC-SCG/Bio-Rad, Hercules, CA) and developed with Amersham Hybond-C (Amersham, Little Chalfont, UK).

**RESULTS**

**Figure 1:** Protein expression assay (PDA) showing detection of protein in the substantia nigra (SN) and putamen in a patient with Parkinson’s disease.

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

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

**Figure 4:** Oligomerization of the A53T variant of α-synuclein by various concentrations of DA, DOPAL, DOPAC, and DOPAL. DOPAL potently oligomerized α-synuclein.

**Figure 5:** Oligomerization of the A53T variant of α-synuclein by various concentrations of DA, DOPAL, DOPAC, and DOPAL. DOPAL potently oligomerized α-synuclein.

**Figure 6:** Oligomerization of α-synuclein with presumed aggregation by DOPAL, such that the protein did not migrate in the gel.

**DISCUSSION**

The main new findings from these experiments are that the catecholaldehyde, DOPAL, potently oligomerizes α-synuclein and that mutation of α-synuclein with the A53T mutant form of the protein results in a substantial amount of non-immigration 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 in vitro, but DOPAL is about 10 times as potent in this regard. The acute metabolites of DOPAH, DOPAC, is less potent than DA.

Planned follow-up studies will extend to cellular models. In rat phaeochromocytoma cells (PC-12), cell manipulations that increase endogenous DOPAL production segment cytotoxic effects of the metabolic stress, mitochrondria (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 oligomerization and β-cell death.

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