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Title of Research *	3,4-DIHYDROXYPHENYLACETALDEHYDE, A DOPAMINE-DERIVED NEUROTOXIN, MODIFIES AND INHIBITS TYROSINE HYDROXYLASE
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Introduction & Purpose *

Parkinson's disease (PD) affects over 1 million individuals in the United States. The disease is characterized by the loss of dopaminergic neurons in the substantia nigra. This leads to a decrease in the neurotransmitter dopamine (DA). Loss of DA leads to tremors, muscle rigidity, and bradykinesia. Dopamine is metabolized by monamine oxidase B to 3,4-dihydroxyphenylacetaldehyde (DOPAL). This metabolite is structurally analogous to DA, but is a reactive intermediate with the potential to interact with proteins and enzymes, causing deleterious effects. It can then be further metabolized to the acid or alcohol by aldehyde dehydrogenase (ALDH) or alcohol reductase (ALR), respectively. There is evidence that DOPAL, at pathological levels, modifies proteins in dopamine neurons. It is hypothesized that the protein modification may yield the inhibition of enzymes that are important to DA biosynthesis, such as tyrosine hydroxylase. Tyrosine hydroxylase (TH) is the enzyme that catalyzes the rate-limiting step in DA synthesis. 3,4-Dihydroxyphenylalanine (L-DOPA) is formed from tyrosine in the presence of the cofactors tetrahydrobiopterin and oxygen. DA is formed through L-DOPA decarboxylation by aromatic L-amino acid decarboxylase. The objectives of this research currently are to 1) measure the toxicity of DOPAL in dopaminergic cell models, 2) to measure the inhibition of tyrosine hydroxylase following DOPAL exposure, and 3) determine the mechanism of inhibition of TH by DOPAL.

Experimental Design *

Materials :Tyrosine and L-DOPA were purchased from Sigma- Aldrich Chemicals. DOPAL was biosynthesized using MAO derived from mouse liver.

PC6-3 cell cultures : PC6-3 cells were cultured by a method previously established (4). No NGF was added (5). Cells were scraped and centrifuged for 1 hr at 100,000 x g. The supernatant was saved; it contained tyrosine hydroxylase from the ruptured cells.

L-DOPA assay using PC6-3 cells : PC6-3 cell lysate (0.05 mg/mL) was incubated in the presence of 100 µM tyrosine and 0.5 mM co-factor tetrahydrobiopterin at 37 °C. A final volume of 500 µL was obtained using 10 mM sodium phosphate buffer (pH=6.4). Aliquots were taken and the reaction was stopped by the addition of 5 µL of perchloric acid. Aliquots were then centrifuged for 3 minutes at 10,000 x g to pellet any precipitated proteins.

Monitoring formation of L-DOPA by HPLC: Formation of L-DOPA was followed using a previously established method (4) with some modifications, including the use of 3% acetonitrile. A C18 capillary column was used. (Standard solutions of L-DOPA, DA, tyrosine and DOPAL. Retention times for the standards were as follows: 5.2, 6.2, 10.3, and 11.1 minutes (DA, L-DOPA, tyrosine, and DOPAL, respectively).

Results *

Dopaminergic cell models show toxicity at concentrations as low as 10 μM DOPAL after only 2 hours. These results indicate that physiologically relevant concentrations of DOPAL are toxic to cells. HPLC analysis of isolated tyrosine hydroxylase incubated with tyrosine and cofactor confirm activity, as measured by L-DOPA production, and DOPAL inhibits TH activity. Concentrations of DOPAL as low as 0.1 μM lead to over 80% inhibition in cell lysate and 3 μM within dopaminergic cells leads to ~41% decrease in TH activity. These results indicate that TH activity is inhibited by even low micromolar DOPAL exposure. Furthermore, DOPAL inhibition appears to be semi-reversible, exhibiting both time and concentration-dependent recovery of enzyme activity.

Conclusions *

These results indicate that low micromolar concentrations of DOPAL are not only toxic to cell models, but also show significant inhibition of tyrosine hydroxylase activity. This is important to Parkinson's disease because as we age, MAO metabolism of DA increases, as well as products of oxidative stress (i.e. 4-HNE, and MDA) increase and have been shown to cause inhibition of ALDH and ALR. This would lead to an increase in DOPAL levels, thus leading to a variety of problems, including protein adduction and inhibition. Tyrosine hydroxylase inhibition would lead to not only a decrease in DA (a hallmark of PD), but because L-DOPA has been shown to have trophic properties, it may lead to a decrease in cell viability as well.

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