HEALTH RESEARCH ABSTRACT SUBMISSIONS #32

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Title of Research *	3,4-Dihydroxyphenylacetaldehyde, an Endogenously Generated Neurotoxin: Metabolism, Toxicity, and Tyrosine Hydroxylase Inhibition
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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-dihyroxyphenylacetaldehyde (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) Determine the metabolism of exogenously applied DOPAL in cell models, and 3) to measure the inhibition of tyrosine hydroxylase following DOPAL exposure.

Experimental Design *

Tyrosine, L-DOPA, dopamine, and DOPAC were purchased from Sigma Aldrich. DOPAL was biosynthesized using rat liver homogenates as previously described in the literature. PC6-3 cell cultures were used as dopamine cell models. Cells were plated in RPMI 1640 medium supplemented with heat-inactivated 10% horse serum, 5% fetal bovine serum, and both penicillin (10 IU/mL) and streptomycin (10 mg/mL). For metabolism and toxicity studies, cells were incubated in the presence of exogenous DOPAL (5-50 µM) for 2 hours, and extracellular media aliquots were taken at time points of 0, 30, 60, 90, and 120 minutes. HPLC-ECD analysis was used to determine metabolism to DOPAC and DOPET. The MTT assay was used to assess mitochondrial function after 2 hours in the presence of exogenously applied DOPAL. To assess tyrosine hydroxylase activity, cell lysate was obtained from PC6-3 cells via sonication and centrifugation in order to obtain the cytosolic fraction. Lysate was incubated in the presence of both tyrosine and co-factor (tetrahydrobiopterin), or tyrosine, cofactor, and DOPAL (0.5-10 µM) for 2.5 hours and aliquots were obtained. HPLC analysis was used to determine the production of L-DOPA over time.

Results *

Dopaminergic cell models show toxicity at concentrations as low as 5 µM DOPAL after only 2 hours. These results indicate that physiologically relevant concentrations of DOPAL are toxic to cells. Furthermore, exogeneously applied DOPAL to PC6-3 cells is taken up and metabolized to both DOPAC and DOPET, via ALDH and ALR, respectively. Results indicate higher concentrations of DOPAL result in more DOPET formation, which lower concentrations result in more DOPAC formation. These results are indicative of the Km values for both ALDH and ALR. HPLC analysis of isolated tyrosine hydroxylase incubated with tyrosine and cofactor confirm activity, as measured by L-DOPA production, and DOPAL inhibits TH activity. These results indicate that TH activity is inhibited by even low micromolar DOPAL exposure.

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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