Published in final edited form as:

Neurochem Res. 2016 September ; 41(9): 2173–2178. doi:10.1007/s11064-016-1959-0.

3,4-Dihydroxyphenylethanol (Hydroxytyrosol) Mitigates the Increase in Spontaneous Oxidation of Dopamine during Monoamine Oxidase Inhibition in PC12 Cells

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Abstract

The catecholaldehyde hypothesis predicts that monoamine oxidase (MAO) inhibition should slow the progression of Parkinson's disease, by decreasing production of the autotoxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL). Inhibiting MAO, however, diverts the fate of cytoplasmic dopamine toward potentially harmful spontaneous oxidation products, indicated by increased 5-S-cysteinyl-dopamine (Cys-DA) levels. 3,4-Dihydroxyphenylethanol (hydroxytyrosol) is an abundant anti-oxidant phenol in constituents of the Mediterranean diet. Whether hydroxytyrosol alters enzymatic or spontaneous oxidation of dopamine has been unknown. Rat pheochromocytoma PC12 cells were incubated with hydroxytyrosol (10 μ M, 180 minutes) alone or with the MAO-A inhibitor clorgyline (1 nM) or the MAO-B inhibitors rasagiline or selegiline (0.5 μ M). Hydroxytyrosol decreased levels of DOPAL by 30% and Cys-DA by 49% ($p < 0.0001$ each). Co-incubation with hydroxytyrosol prevented the increases in Cys-DA seen with all 3 MAO inhibitors. Hydroxytyrosol therefore inhibits both enzymatic and spontaneous oxidation of endogenous dopamine and mitigates the increase in spontaneous oxidation during MAO inhibition.

Keywords

Hydroxytyrosol; DOPET; DOPAL; Cysteinyl-dopamine; Monoamine oxidase; Parkinson's disease

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AUTHORSHIP CONTRIBUTIONS

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CONFLICT OF INTEREST

None of the authors has a conflict of interest to report.

Dr. Goldstein is Chair of the Education Committee and sits on the Board of Directors of the American Autonomic Society, under an approved Outside Activity. For these services he receives no payment in cash or kind.

Intra-neuronal enzymatic metabolism of the neurotransmitter, dopamine, passes through the intermediate metabolite, 3,4-dihydroxyphenylacetaldehyde (DOPAL, Fig. 1). According to the catecholaldehyde hypothesis, accumulation of DOPAL contributes to the loss of dopaminergic neurons in Parkinson's disease. Inhibition of monoamine oxidase (MAO), by decreasing DOPAL production, should therefore slow the neurodegeneration [1]. MAO inhibition, however, secondarily builds up cytoplasmic dopamine, resulting in increased spontaneous oxidation to dopamine-quinone [2, 3] and formation of potentially toxic compounds [4–7], including 5-S-cysteinyl-dopamine (Cys-DA) [8–11]. Harmful effects of augmented spontaneous dopamine oxidation during MAO inhibition might offset the beneficial effects of decreasing DOPAL production. It is reasonable to suggest anti-oxidant treatment as an adjuvant could mitigate the secondary increase in dopamine oxidation in this setting.

3,4-Dihydroxyphenylethanol (hydroxytyrosol, DOPET), a major phenolic compound in olive oil [12] and red wine [13, 14], is an important constituent of the Mediterranean diet. Neurochemical properties of DOPET suggest that it could enhance the neuroprotective efficacy of MAO inhibitor treatment. Because DOPET is a neutral alcohol, exogenously administered DOPET would be expected to diffuse readily within the total body water space and enter central neurons; and because DOPET is a catechol, intracellular DOPET would be expected to act as an anti-oxidant. Consistent with these expectations, oral administration of DOPET to rats results in dose-related increases in brain tissue levels of DOPET and its metabolites [15], and after systemic injection DOPET is detected in striatal microdialysate [16]. Systemically administered DOPET prevents the increases in lipid peroxides and the decreases in reduced glutathione levels in striatum that are evoked by 3-nitropropionic acid [17], indicating an ability to exert anti-oxidant effects in central dopaminergic neurons. Moreover, intracellular DOPET inhibits tyrosine hydroxylase (TH) [18], and by decreasing the rate of dopamine synthesis DOPET could decrease the rate of spontaneous oxidation of cytoplasmic dopamine and consequently attenuate Cys-DA production.

The purpose of this study was to determine whether DOPET mitigates the MAO inhibitor-induced increase in spontaneous dopamine oxidation as indicated by increased Cys-DA levels, without impeding the MAO inhibitor-induced decrease in DOPAL production. PC12 cells were used, since they are known to produce dopamine, DOPAL, and Cys-DA endogenously and exhibit increased Cys-DA production during MAO inhibition [19]. The cells were incubated with the MAO-A inhibitor clorgyline or the MAO-B inhibitors rasagiline or selegiline, with or without DOPET co-incubation. From the processes depicted in Fig. 1 we predicted that DOPET would decrease levels of both DOPAL and Cys-DA and that co-incubation of DOPET with an MAO inhibitor would result in less Cys-DA production than that observed with the MAO inhibitor alone.

METHODS

Cells and Reagents

PC12 cells were from the American Type Culture Collection (ATCC, Manassas, VA; PC12 cells catalog no. CRL-1721); F12K cell culture medium from Gibco Life Technologies (Grand Islands, NY); tolcapone (to block catechol-O-methyltransferase) from Orion Pharma

(Espoo, Finland); DOPAL standard from Santa Cruz Biotechnology, Inc. (Dallas, TX); and Cys-DA standard from the NIMH Chemical Synthesis and Drug Supply Program (No. C-805).

Non-adherent, non-differentiated cells PC12 cells were kept frozen in liquid nitrogen until passaged for experiments. The cells were grown in F12K medium with 15% horse serum and 2.5% fetal bovine serum and incubated at 37 °C in an atmosphere of 5% carbon dioxide. The medium was replaced several times per week and cells passaged once per week.

At 24 hours prior to plating for experiments, the cells were centrifuged and the medium was replaced with medium containing 10 mM tolcapone. After the 24 hours, the cells were collected, suspended in the same medium, and counted (Cellometer, Nexcelom Bioscience, Lawrence, MA). About 500,000 cells/well were plated in 12-well plates. The experiments began after 24 hours of incubation in tolcapone-containing medium.

Experiments

There were 4 experimental treatments—control (no added drug), DOPET (10 μ M), an MAO inhibitor alone (1 nM for clorgyline, 500 nM for rasagiline or selegiline), and DOPET +MAO inhibitor. DOPET and the MAO inhibitor were added to the incubation medium approximately simultaneously, and the cells were incubated at 37 °C for 3 hours. In one experiment, DOPET and rasagiline were used (4 replicates for each of the 4 conditions). In a second experiment, DOPET and clorgyline or selegiline were used (4 replicates, 4 conditions, 2 drugs).

Assay Methods

The cells and medium were assayed for contents of catechols by batch alumina extraction followed by liquid chromatography with electrochemical detection in our laboratory [20]. Levels in the cells and medium were summed and expressed as pmol/well.

Indices of Tyrosine Hydroxylase Activity

To determine whether DOPET, MAO inhibition, or the combination of DOPET with an MAO inhibitor decreases TH activity, we summed the contents of endogenous dopamine and its metabolites and assayed endogenous 3,4-dihydroxyphenylalanine (DOPA). Under steady state conditions the rate of accumulation of DOPA equals the rate of DOPA synthesis due to intracellular TH activity [20].

Data Analysis and Statistics

Mean values were expressed $\pm$ 1 standard error of the mean. Statistical testing utilized KaleidaGraph 4.5 (Synergy Software, Reading, PA). Data for levels of catechols across treatments were compared by factorial analyses of variance, with post-hoc comparisons among the treatments by Fisher's Least Significant Difference post-hoc test and comparisons with control values by Dunnett's post-hoc test.

RESULTS

The MAO inhibitors markedly decreased levels of DOPAL and of 3,4-dihydroxyphenylacetic acid (DOPAC), while increasing Cys-DA levels ($p<0.0001$ each; Fig. 2).

Across the 2 experiments ($N=8$), incubation with DOPET decreased DOPAL levels by 30% and Cys-DA levels by 49% ($p<0.0001$ each; Fig. 2). DOPAL levels were decreased to a lesser extent than found with MAO inhibition. DOPET also decreased the sum of dopamine and its metabolites and decreased DOPA levels ($p<0.0001$ each; Fig. 3).

Co-incubation with DOPET and MAO inhibitors markedly attenuated (rasagiline) or abolished (clorgyline, selegiline) the increases in Cys-DA levels seen with the inhibitors alone (Fig. 2). With the co-incubation, Cys-DA levels were even below those in the control untreated cells ($p=0.0005$ for rasagiline, $p<0.0001$ for clorgyline or selegiline).

The sum of dopamine and its metabolites was lower with the co-incubation than with the MAO inhibitors or DOPET alone (Fig. 3). For all three MAO inhibitors, DOPA levels were also lower when the cells were co-incubated with DOPET and the MAO inhibitor than when the cells were incubated with the MAO inhibitor alone ($p<0.0001$ for clorgyline, $p=0.01$ each for rasagiline and selegiline).

DISCUSSION

In this study the MAO inhibitors clorgyline, rasagiline, and selegiline decreased endogenous levels of the autotoxic dopamine metabolite DOPAL in PC12 cells but concurrently increased spontaneous oxidation of dopamine as indicated by Cys-DA levels, confirming the findings in a recent study [11]. The main new findings reported here are that DOPET decreases DOPAL and Cys-DA levels and prevents the increases in Cys-DA levels seen with MAO inhibition.

Mechanisms of Attenuation of Cys-DA Production by DOPET

DOPET incubation decreased the sum of dopamine and its metabolites (excluding DOPET itself) and decreased the concentration of DOPA, the immediate product of the TH step (Fig. 3). These data indicate DOPET-induced negative feedback inhibition of dopamine synthesis and consequently decreased spontaneous oxidation of dopamine.

DOPET-induced diminution of TH activity would be expected to decrease the baseline level of Cys-DA but would not be expected to attenuate the MAO inhibitor-induced increases in Cys-DA from the diminished level. The fact that DOPET not only decreased Cys-DA levels but also severely attenuated (rasagiline) or completely prevented (clorgyline, selegiline) the increase in Cys-DA levels during MAO inhibition compared to the value with DOPET alone indicates that DOPAL's anti-oxidant property slows the conversion of dopamine to dopamine-quinone and thereby to Cys-DA.

Dual Effect of MAO Inhibition on Cys-DA Production

Considering that all three MAO inhibitors increased Cys-DA levels while decreasing values for the indices of TH activity, MAO inhibition seems to exert a dual effect on the spontaneous oxidation of dopamine. The main effect is stimulatory, due to inhibition of metabolism of cytoplasmic dopamine by MAO; however, this is modulated somewhat by an inhibitory effect due to feedback inhibition of TH by built up cytoplasmic dopamine. The net effect is to increase cytoplasmic dopamine, which in turn oxidizes spontaneously to form Cys-DA.

Therapeutic Implications

The present results have clear implications for strategies to slow the progression of diseases such as Parkinson's disease that involve loss of catecholaminergic neurons. MAO inhibition effectively decreases production of autotoxic DOPAL, but as confirmed here this treatment increases spontaneous oxidation of dopamine. One may limit this secondary effect via an anti-oxidant such as hydroxytyrosol. The present results justify clinical studies to determine the plasma and central nervous system bioavailability of exogenous hydroxytyrosol, the effects of MAO inhibition on cerebrospinal fluid Cys-DA levels, and the ability of hydroxytyrosol to mitigate the increase in cerebrospinal fluid Cys-DA during MAO inhibitor treatment. We view these as necessary first steps toward a clinical trial to test whether hydroxytyrosol combined with an MAO inhibitor slows the catecholaminergic neurodegeneration in Parkinson's disease.

Acknowledgments

The research reported here was supported by the intramural research program of the National Institute of Neurological Disorders and Stroke.

Abbreviations

ALDH	aldehyde dehydrogenase
DA	dopamine
DHPG	3,4-dihydroxyphenylglycol
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPET	3,4-dihydroxyphenylethanol
NE	norepinephrine
PD	Parkinson disease
VMAT	vesicular monoamine transporter

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Fig. 1. Concept Diagram Showing Effects of Monoamine Oxidase (MAO) Inhibition and 3,4-Dihydroxyphenylethanol (DOPET) on Endogenous 5-S-Cysteinyl-Dopamine (Cys-DA) Production

Dihydroxyphenylacetaldehyde (DOPAL) is formed from the action of monoamine oxidase (MAO) in the outer mitochondrial membrane on cytoplasmic dopamine (DA). MAO inhibition builds up cytoplasmic DA, resulting in spontaneous oxidation to DA-quinone (DA-Q) and then Cys-DA. Cytoplasmic DA buildup also increases vesicular uptake via the vesicular monoamine transporter (VMAT) and consequently increases the synthesis of norepinephrine (NE) and increases constitutive release of DA and NE. As indicated by the red arrows, cytoplasmic DA buildup also feedback inhibits tyrosine hydroxylase (TH), decreasing production of DOPA. DOPET inhibits TH and decreases endogenous dopamine synthesis. In addition, DOPET interferes with the spontaneous oxidation of dopamine to dopamine-quinone (DA-Q). Abbreviations: AR=aldehyde/aldose reductase; LAAAD=L-aromatic-amino-acid decarboxylase; TYR=tyrosine.

Fig. 2. Mean ($\pm$ SEM) Concentrations of Catechols (pmoles per 1 mL well) during Incubation with DOPET or MAO Inhibitors

Conditions: No added drug (CON), DOPET, rasagiline (R), clorgyline (C), selegiline (S), DOPET+R, DOPET+C, and DOPET+S. One experiment (panels A and C) involved CON, DOPET, R, and DOPET+R, with $n=4$ replicates for each condition; and another experiment (panels B and D) involved CON, DOPET, C, S, DOPET+C and DOPET+S, with $n=4$ replicates for each condition. Horizontal dashed lines placed to emphasize Cys-DA levels above CON in the setting of MAO inhibitors and below CON in the setting of co-incubation with DOPET and MAO inhibitors. (****) significantly different from CON by Dunnett's post-hoc test, with $p<0.0001$; (***) $p<0.001$.

Fig. 3. Mean ($\pm$ SEM) Concentrations of the Sum of Dopamine and Its Metabolites or Concentrations of DOPA (pmoles per 1 mL well) during Incubation with DOPET or MAO Inhibitors

Conditions: No added drug (CON), DOPET, rasagiline (R), clorgyline (C), selegiline (S), DOPET+R, DOPET+C, and DOPET+S. One experiment (panels A and B) involved CON, DOPET, R, and DOPET+R, $n=4$ replicates for each condition; and another experiment (panels C-D) involved CON, DOPET, C, S, DOPET+C and DOPET+S, $n=4$ replicates for each condition. Panels A and C show stack diagrams to depict the sum of DA, DOPAC, DOPAL, norepinephrine (NE), Cys-DA, and 3,4-dihydroxyphenylglycol (DHPG). Panels B and D show DOPA levels. (****) significantly different from CON by Dunnett's post-hoc test, with $p<0.0001$; (***) $p<0.001$; (**) $p<0.01$; (*) $p<0.05$. DOPET alone decreased dopamine and the sum of its metabolites and decreased DOPA levels. Co-incubation of DOPET with MAO inhibitors produced further decreases in the sum of dopamine and its metabolites and in DOPA levels.