Comparison of Monoamine Oxidase Inhibitors in Decreasing Production of the Autotoxic Dopamine Metabolite 3,4-Dihydroxyphenylacetaldehyde in PC12 Cells

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ABSTRACT

According to the catecholaldehyde hypothesis, the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL) contributes to the loss of nigrostriatal dopaminergic neurons in Parkinson’s disease. Monoamine oxidase-A (MAO-A) catalyzes the conversion of intraneuronal dopamine to DOPAL and may serve as a therapeutic target. The “cheese effect”—paroxysmal hypertension evoked by tyramine-containing foodstuffs—limits clinical use of irreversible MAO-A inhibitors. Combined MAO-A/B inhibition decreases DOPAL production in rat pheochromocytoma PC12 cells, but whether reversible MAO-A inhibitors or MAO-B inhibitors decrease endogenous DOPAL production is unknown. We compared the potencies of MAO inhibitors in attenuating DOPAL production and examined possible secondary effects on dopamine storage, constitutive release, synthesis, and auto-oxidation. Catechol concentrations were measured in cells and medium after incubation with the irreversible MAO-A inhibitor clorgyline, three reversible MAO-A inhibitors, or the MAO-B inhibitors selegiline or rasagiline for 180 minutes. Reversible MAO-A inhibitors were generally ineffective, whereas clorgyline (1 nM), rasagiline (500 nM), and selegiline (500 nM) decreased DOPAL levels in the cells and medium. All three drugs also increased dopamine and norepinephrine, decreased 3,4-dihydroxyphenylalanine, and increased cysteinyl-dopamine concentrations in the medium, suggesting increased vesicular uptake and constitutive release, decreased dopamine synthesis, and increased dopamine spontaneous oxidation. In conclusion, clorgyline, rasagiline, and selegiline decrease production of endogenous DOPAL. At relatively high concentrations, the latter drugs probably lose their selectivity for MAO-B. Possibly offsetting increased formation of potentially toxic oxidation products and decreased formation of DOPAL might account for the failure of large clinical trials of MAO-B inhibitors to demonstrate slowing of neurodegeneration in Parkinson’s disease.

Introduction

The movement disorder in Parkinson’s disease is associated with profound striatal dopamine (DA) depletion (Kish et al., 1988), which results from loss of nigrostriatal dopaminergic terminals and deficient vesicular DA storage in the remaining terminals (Goldstein et al., 2013; Pifl et al., 2014).

To explain the vulnerability of catecholamine neurons in Parkinson’s disease, we have proposed “autotoxicity”—inherent cytotoxicity of catecholamine metabolites (Goldstein et al., 2014)—which is probably a contributory pathogenetic mechanism. In neurons, cytoplasmic DA is converted to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by monoamine oxidase-A (MAO-A; Fig. 1). DOPAL is toxic (Panneton et al., 2010) via generation of reactive oxygen species (Anderson et al., 2011), inhibition of mitochondrial functions (Berman and Hastings, 1999), and protein modifications (Burke et al., 2003). According to the catecholaldehyde hypothesis, DOPAL causes or contributes to the nigrostriatal neurodegeneration in Parkinson’s disease (Burke et al., 2003).

Post-mortem neurochemical studies documenting increased putamen DOPAL (Goldstein et al., 2013) are consistent with the catecholaldehyde hypothesis, but this does not imply that DOPAL buildup in Parkinson’s disease is pathogenic. A way to test the catecholaldehyde hypothesis would be to determine if inhibiting MAO-A slows catecholaminergic neurodegeneration, as assessed by neuroimaging (Goldstein et al., 2007) or neurochemical (Goldstein et al., 2012a) biomarkers. Patients taking MAO-A inhibitors, however, are at risk of toxicity from the “cheese effect,” dangerously high blood pressure from ingesting tyramine-containing foodstuffs.

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ABBREVIATIONS: Cys-DA, 5-S-cysteinyl-dopamine; DA, dopamine; DHPG, 3,4-dihydroxyphenylglycol; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPA, 3,4-dihydroxyphenylalanine; DOPAL, 3,4-dihydroxyphenylacetaldehyde; DOPET, 3,4-dihydroxyphenylethanol; MAO-A, monoamine oxidase-A; MAO-B, monoamine oxidase-B; NE, norepinephrine.
(Horwitz et al., 1964). MAO-B inhibitors pose little or no risk of a cheese effect (Elsworth et al., 1978). Meanwhile, at relatively high but clinically used doses, the MAO-B inhibitor selegiline decreases plasma levels of MAO-A metabolites (Eisenhofer et al., 1986). In formulations that circumvent first-pass metabolism in the liver, selegiline inhibits MAO-A in the human brain (Fowler et al., 2015). Reversible MAO-A inhibitors have been under development, because they are less likely than irreversible MAO-A inhibitors to increase the risk of a cheese effect (Youdim and Weinstock, 2004).

No previous studies have compared the different types of MAO inhibitors in terms of their abilities to attenuate endogenous DOPAL production. Making these comparisons was the primary purpose of the present study.

We also wished to explore potential secondary effects of MAO inhibition, because these effects might augment or mitigate the benefits of decreasing DOPAL production. Enzymatic deamination and vesicular uptake are alternative means of disposition of cytoplasmic DA (Fig. 1). Possible beneficial effects of MAO inhibition include augmented vesicular storage and, consequently, increased constitutive release of DA. There is indirect evidence that MAO inhibitor treatment enhances vesicular uptake of cytoplasmic catecholamines (Raffel and Wieland, 1999; Lohr et al., 2014), and animals with increased efficiency of vesicular uptake have augmented constitutive dopamine release (Geller et al., 1993; Sun et al., 2004; Lohr et al., 2014). Cellular DA content is mainly within vesicles (Mosharov et al., 2006), and since norepinephrine (NE) is produced exclusively within vesicles by the action of DA-β-hydroxylase acting on DA taken up from the cytoplasm, enhanced vesicular uptake would be expected to increase cell contents of endogenous DA and NE. MAO inhibition may also act indirectly to decrease DA synthesis via enhanced feedback inhibition exerted by accumulation of DA in the cytoplasm (Ames et al., 1978; Laschinski et al., 1986; Gordon et al., 2008). Since DOPA is the immediate product of enzymatic hydroxylation of tyrosine, decreased tyrosine hydroxylase activity would be expected to attenuate DOPA production (Goldstein et al., 1987; Kvetnansky et al., 1992).

Large multicenter clinical trials have assessed whether MAO-B inhibitors slow the neurodegenerative process in Parkinson’s disease, and the results have been disappointing (de la Fuente-Fernandez et al., 2010; Hofeld and Nutt, 2011). A potential explanation for the less-than-hoped-for results of MAO inhibition is secondary buildup of cytoplasmic DA and, consequently, augmented spontaneous oxidation of DA to form DA-quinone, which might offset beneficial effects of decreased DOPAL production. DA-quinone is toxic via spontaneous conversion to dopaminochrome (Linsenbardt et al., 2009; Aguirre et al., 2012) and reactivity with glutathione or cysteine to form 5-S-cysteinyl-dopamine (Cys-DA) (Montine et al., 1997; Spencer et al., 2002). Cys-DA provides a biomarker of DA auto-oxidation (Fernstadt et al., 1986; Carlsson and Fernstadt, 1991). In guinea pigs, MAO inhibition increases striatal and limbic system tissue contents of Cys-DA (Fernstadt and Carlsson, 1991). Another purpose of this study was to assess the effects of MAO inhibition on endogenous DOPAL and Cys-DA levels.

The experiments reported here were carried out in rat pheochromocytoma PC12 cells. PC12 cells synthesize, store, release, and metabolize endogenous catecholamines, and they produce and metabolize DOPAL (Lamensdorf et al., 2000). Most of the MAO activity in PC12 cells is from MAO-A (Lenzen et al., 1987), the type present in dopaminergic neurons. As shown here for the first time, PC12 cells also produce endogenous Cys-DA. The ability to measure endogenous DOPAL and Cys-DA simultaneously provided a unique opportunity to determine whether, at concentrations that effectively decrease DOPAL production, MAO inhibition affects DA auto-oxidation as indicated by Cys-DA levels.

If it were found that MAO-B inhibition effectively reduced DOPAL production, this would leave open the question of which MAO-B inhibitor to test in a clinical trial. As noted earlier, selegiline, at clinically used doses, can decrease...
MAO-A activity in the brain and periphery (Eisenhofer et al., 1986; Fowler et al., 2015). Whether rasagiline, the other currently approved MAO-B inhibitor, decreases indices of MAO-A activity has been unknown. Therefore, in this study, we included data about plasma levels of catechols from healthy volunteers and from parkinsonian patients who were on the currently used dose of rasagiline (1 mg/day) or were not on rasagiline at the time of evaluation at the National Institutes of Health Clinical Center.

Materials and Methods

Institutional approval of the research reported here was obtained from the Division of Intramural Research of the National Institute of Neurological Disorders and Stroke. The human studies were conducted at the National Institutes of Health Clinical Center after giving informed consent to participate in protocols approved by the National Institute of Neurological Disorders and Stroke Institutional Review Board.

Materials. Rat pheochromocytoma PC12 cells were from the American Type Culture Collection (Manassas, VA). The cell culture medium, F12K, was from Gibco Life Technologies (Grand Islands, NY); tolcapone (to block catechol-O-methyltransferase) from Orion Pharma (Espoo, Finland); and dopamine, norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenylethanol (DOPET), and 3,4-dihydroxyphenylglycol (DHPG) from Sigma-Aldrich (St. Louis, MO). DOPAL standard was synthesized in the laboratory of and provided by Dr. Kenneth L. Kirk (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) or was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX). Cysteinyl-DA standard was obtained from the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (No. C-805; National Institute of Mental Health, Bethesda, MD).

A total of seven MAO inhibitors were compared in terms of effects on DOPAL production. Moclobemide, minaprine, tolcapone, clorgyline hydrochloride, pargyline hydrochloride, selegiline hydrochloride, and rasagiline mesylate were obtained from Sigma-Aldrich (St. Louis, MO).

PC12 Cell Cultures. PC12 cells were kept frozen in liquid nitrogen until passaged for experiments. The experiments reported here involved nonadherent, nondifferentiated cells. Nonadherent PC12 cells were grown in F12K medium with 15% horse serum and 2.5% fetal bovine serum. The cells were incubated at 37°C in an atmosphere of 5% carbon dioxide. Media were changed 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 μM tolcapone. Since PC12 cells express catechol-O-methyltransferase, whereas dopaminergic neurons do not, the cells in this study were always preincubated for 24 hours in medium containing the catechol-O-methyltransferase inhibitor, tolcapone. After the 24 hours, the cells were collected, suspended in the same medium, and counted using a Cellometer device (Nexcelom Bioscience, Lawrence, MA). About 500,000 cells/well were plated in 12-well plates.

The experiments began after 24 hours of incubation with tolcapone-containing medium.

Preliminary Experiments. Preliminary experiments were done to select MAO inhibitors for comprehensive concentration-response and comparative efficacy studies. In a preliminary concentration-response experiment (preliminary experiment A), five MAO inhibitors were compared—the reversible MAO-A inhibitors minaprine (30 μM, N = 2–3), moclobemide (30 μM, N = 3), and tolcapone (30 μM, N = 3); the MAO-B inhibitor pargyline (10 μM, N = 2–3); and the irreversible MAO-A inhibitor clorgyline (10 μM, N = 2). Among the reversible MAO-A inhibitors, tolcapone seemed most promising, and so another experiment (preliminary experiment B) involved various concentrations of tolcapone (0, 3, 10, 30, and 100 μM, N = 4 at each concentration) compared with pargyline (10 μM, N = 2) and clorgyline (10 μM, N = 2). To explore the potency of an MAO-B inhibitor in decreasing DOPAL production, another experiment (preliminary experiment C) involved various concentrations of the MAO-B inhibitor selegiline compared with clorgyline (0, 0.001, 0.010, 0.1, 1.0, and 10 μM, N = 2 at each concentration).

Comparisons of Clorgyline, Rasagiline, and Selegiline. Based on the preliminary experiments, more complete experiments were conducted with the MAO-A inhibitor clorgyline and the MAO-B inhibitors rasagiline and selegiline. Comparisons between clorgyline, rasagiline, and selegiline took two forms. First, we conducted concentration-response experiments. A typical concentration-response experiment was done using 18-well plates, 1 ml per well. Six microliters (final concentration 0.6% v:v) of dimethylsulfoxide vehicle was added into three wells. In a total of 12 other wells, dimethylsulfoxide vehicle containing various concentrations of MAO inhibitor (two to three wells at each concentration) was added. For all 18 wells, at 180 minutes, the cells and medium were separated and frozen until assayed for catechols.

Since the preliminary experiments indicated that clorgyline is at least 2 orders of magnitude more potent than selegiline, the concentration-response experiments were across different concentration ranges. Clorgyline concentrations were 0.001, 0.01, 0.1, 1, and 100 nM (N = 2 at each concentration), and selegiline concentrations were 0.01, 1, 10, 100, 1000, and 10,000 nM (N = 2 at each concentration). There were two rasagiline concentration-response experiments. In one experiment, rasagiline concentrations were 0.01, 0.1, 1, 10, and 100 nM (N = 3 at each concentration) and 1000 nM (N = 2, as one data set was lost for technical reasons). In a second concentration-response experiment, rasagiline concentrations were 10, 100, 1000, and 10,000 nM (N = 3 at each concentration). For constructing concentration-response curves, the values at each concentration in each experiment were expressed as fractions of the values with vehicle control in that experiment.

Based on the concentration-response curves, a final experiment was done comparing clorgyline, rasagiline, and selegiline (N = 6 for each drug) at drug concentrations identified as effective in reducing DOPAL levels in the concentration-response experiments by at least 50%. For the final experiment, the clorgyline concentration was 1 nM, and the rasagiline and selegiline concentrations were 500 nM.

Catechol Assays. Catechols were assayed by liquid chromatography with electrochemical detection after batch alumina extraction (Eisenhofer et al., 1986; Goldstein et al., 2012b). The analyses were done on matching liquid chromatographic/electrochemical detection systems. Each chromatographic system included a Waters 515 pump and 717 Plus refrigerated autosampler (Waters, Inc., Milford, MA). The mobile phase was as follows: 11:1 of type I water, 13.8 g of NaH₂PO₄, 50 mg of EDTA, 59 mg of octanesulfonic acid, acetonitrile approximately 2.5%, pH approximately 3.2 (acetonitrile and pH adjusted to optimize chromatographic separations). The column was a Thomson BioAdvantage Basic C18 5 μM 250 × 4.6 mm (part no. BA400-046250; Thomson Instrument Co., Clear Brook, VA). The postcolumn electrochemical detector system included an ESA Biosciences (now Thermo) Coulomac III controller and a series of Conditioning Cell (part no. 70-6088) and then an Analytical Cell (part no. 70-5561). The Conditioning Cell electrode potential was set at +350 mV, and the Analytical Cell electrodes were set at +150 mV and -350 mV.

Cell contents of catechols were assayed from 100- to 200-μl aliquots of the 400-μl of disrupted cells from each 1 ml well. Medium contents were assayed from aliquots of aspirated incubation medium. The contents of catechols in the cells and medium were expressed in units of picomoles per well.

Plasma Catechols in Patients on Rasagiline. Clinical data about plasma catechols were reviewed from patients with parkinsonism and from healthy volunteers. Patients on rasagiline were taking the drug at 1 mg/day for clinical indications. The plasma was assayed for catechols including DOPAC, the main MAO-A metabolite of DA,
and DHPG, the main MAO-A metabolite of NE in our laboratory. The used methodology (Holmes et al., 1994) was based on that used previously by our group to document MAO-A inhibition by selegiline (Eisenhofer et al., 1986). To avoid artifactual effects of levodopa treatment, neurochemical data were included only from patients with plasma DOPA levels less than 3,000 pg/mL (about 15 pmol/mL). Data from patients on selegiline were excluded. There were 34 parkinsonian patients who met the criteria for data inclusion—seven on rasagiline and 27 not on rasagiline—and 52 healthy volunteer control subjects.

Data Analysis and Statistics. For constructing concentration-response curves, the data on levels of analytes were lumped at each of the various drug concentrations across all the experiments.

Neurochemical data were displayed using KaleidaGraph 4.01 (Synergy Software, Reading, PA). Factorial analyses of variance with Fisher’s PLSD post-hoc test were used to compare clorgyline, rasagiline, and selegiline in the final $N = 6$ study. In carrying out statistical analyses of clinical data for plasma levels of catechols, since standard deviations varied with the mean values, the factorial analyses of variance were conducted on log-transformed data to ensure homogeneity of variance. A $P$ value less than 0.05 defined statistical significance.

Results

The liquid chromatographic-electrochemical method used in this study successfully baseline-resolved eight endogenous catechols of interest—the deaminated metabolites DOPAL, DOPAC, DOPET, and DHPG; the catecholamines DA and NE; the catecholamine precursor DOPA; and the DA auto-oxidation product Cys-DA (Fig. 2). Because of the wide range of catechol concentrations in cells and medium, from 80 fmol/ml (cellular DHPG) to more than 10 nmol/ml (cellular DA), multiple volumes were assayed and alumina eluates injected for each sample, and each chromatographic run lasted a relatively long time (typically 2 hours). The DOPAL peak stood out from others in being short and broad, which is a characteristic feature of this aldehyde (Goldstein et al., 2012b). Figure 2 also demonstrates that the assay method enabled simultaneous measurements of endogenous DOPAL and Cys-DA levels in the incubation medium and illustrates that clorgyline decreases levels of DOPAL and other deaminated metabolites.

Preliminary Experiments. In a preliminary experiment (Table 1), we compared five MAO inhibitors [minaprine (30 μM), moclobemide (30 μM), toloxatone (30 μM), pargyline (10 μM), and clorgyline (10 μM)] in terms of medium contents of catechols after 180 minutes of incubation ($N = 2–3$ replicates per drug). Neither minaprine nor moclobemide decreased levels of DOPAL or of other deaminated metabolites, and neither was considered further. Toloxatone produced moderate decreases, whereas pargyline and especially clorgyline produced large decreases despite lower concentrations (Table 1). In another experiment, incubation of PC12 cells with toloxatone ($N = 4$ each at 0, 3, 10, 30, and 100 μM) concentration-dependently decreased medium concentrations of DOPAL, DOPAC, DOPET, and DHPG; increased DA and NE; and decreased DOPA levels ($P < 0.0001$ for 3 vs. 0 μM for each analyte by independent-means $t$ tests); however, toloxatone required relatively high drug concentrations to exert these effects, whereas clorgyline stood out in terms of potency. Toloxatone was not considered further.

In the third preliminary experiment ($N = 4$ for each drug at 0.1, 1, and 10 μM; Table 1), clorgyline, rasagiline, and selegiline all decreased medium DOPAL, DOPAC, DOPET, and DHPG levels, again with clorgyline standing out. Based on the results of the preliminary experiments, clorgyline, rasagiline, and selegiline were selected and compared further in more complete experiments involving larger concentration ranges or numbers of replicates.

Clorgyline versus Rasagiline versus Selegiline. In the concentration-response experiments (Fig. 3; Tables 2 and 3), clorgyline was by far more potent than the other MAO inhibitors. A 50% decrease in medium DOPAL content was attained at about a clorgyline concentration of about 1 nM.
which was about 200 times lower than the concentration for rasagiline and 600 times lower than that for selegiline.

Analogous results were obtained for cell contents of the analytes of interest (Table 3); however, several data points were missing because of interfering chromatographic peaks or undetectable cell contents.

At high clorgyline concentrations, medium concentrations of DA, NE, and Cys-DA changed biphasically (Fig. 3), and

![Graphs of DOPAL, DOPAC, DOPET, and DHPG](image1)

![Graphs of DA, NE, DOPA, and Cys-DA](image2)

**Fig. 3.** Effects of various concentrations of clorgyline, rasagiline, and selegiline on medium contents of catechols at 180 minutes of incubation. Shown are the mean ± S.E.M. levels and the number of data points at each concentration. Dashed lines indicate drug concentrations associated with a 50% decrease in medium DOPAL. Clorgyline (green), rasagiline (blue), and selegiline (red) produced concentration-dependent decreases in medium contents of deaminated metabolites and DOPA and increases in medium contents of DA, NE, and Cys-DA. Clorgyline was much more potent than rasagiline, selegiline, or toloxatone (brown) in all the effects on levels of catechols. At high clorgyline concentrations, medium concentrations of DA, NE, and Cys-DA changed biphasically (Fig. 3), and

<table>
<thead>
<tr>
<th>Drug</th>
<th>DOPAL</th>
<th>DOPAC</th>
<th>DOPET</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3)</td>
<td>137 ± 2</td>
<td>743 ± 37</td>
<td>726 ± 15</td>
<td>24.9 ± 1.0</td>
</tr>
<tr>
<td>Minaprine, 30 μM (2)</td>
<td>366 ± 25</td>
<td>628 ± 115</td>
<td>801 ± 8</td>
<td>23.1 ± 1.9</td>
</tr>
<tr>
<td>Moclobemide, 30 μM (3)</td>
<td>130 ± 4</td>
<td>591 ± 22</td>
<td>782 ± 7</td>
<td>21.3 ± 0.4</td>
</tr>
<tr>
<td>Toloxatone, 30 μM (3)</td>
<td>58 ± 1</td>
<td>253 ± 19</td>
<td>358 ± 7</td>
<td>15.7 ± 0.4</td>
</tr>
<tr>
<td>Pargyline, 10 μM (3)</td>
<td>27 ± 3</td>
<td>176 ± 6</td>
<td>286 ± 5</td>
<td>14.5 ± 0.3</td>
</tr>
<tr>
<td>Clorgyline, 10 μM (3)</td>
<td>6.6 ± 0.0</td>
<td>125 ± 9</td>
<td>188 ± 1</td>
<td>9.6 ± 0.5</td>
</tr>
<tr>
<td>Control (4)</td>
<td>173 ± 1</td>
<td>510 ± 16</td>
<td>311 ± 10</td>
<td>8.9 ± 0.2</td>
</tr>
<tr>
<td>Toloxatone, 3 μM (4)</td>
<td>69 ± 1</td>
<td>228 ± 5</td>
<td>127 ± 5</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>Toloxatone, 10 μM (4)</td>
<td>47 ± 1</td>
<td>155 ± 4</td>
<td>87 ± 1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Toloxatone, 30 μM (4)</td>
<td>21 ± 0</td>
<td>77 ± 2</td>
<td>56 ± 2</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>Toloxatone, 100 μM (4)</td>
<td>19 ± 0</td>
<td>47 ± 2</td>
<td>48 ± 2</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Pargyline, 10 μM (2)</td>
<td>16 ± 1</td>
<td>52 ± 0</td>
<td>57 ± 2</td>
<td>3.8 ± 0.0</td>
</tr>
<tr>
<td>Clorgyline, 10 μM (2)</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
<td>0 ± 0</td>
<td>11.3 ± 1.4</td>
</tr>
<tr>
<td>Control (4)</td>
<td>83 ± 2</td>
<td>463 ± 15</td>
<td>307 ± 8</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>Pargyline, 100 nM (4)</td>
<td>21 ± 1</td>
<td>179 ± 10</td>
<td>141 ± 5</td>
<td>8.7 ± 0.3</td>
</tr>
<tr>
<td>Pargyline, 1 μM (4)</td>
<td>20 ± 1</td>
<td>122 ± 2</td>
<td>139 ± 3</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Pargyline, 10 μM (4)</td>
<td>14 ± 1</td>
<td>89 ± 5</td>
<td>92 ± 3</td>
<td>6.9 ± 0.1</td>
</tr>
<tr>
<td>Rasagiline, 100 nM (4)</td>
<td>29 ± 1</td>
<td>120 ± 1</td>
<td>136 ± 2</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Rasagiline, 1 μM (4)</td>
<td>24 ± 1</td>
<td>90 ± 3</td>
<td>104 ± 1</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>Rasagiline, 10 μM (4)</td>
<td>19 ± 1</td>
<td>71 ± 2</td>
<td>77 ± 3</td>
<td>6.1 ± 0.3</td>
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<tr>
<td>Clorgyline, 100 nM (4)</td>
<td>9 ± 1</td>
<td>59 ± 1</td>
<td>41 ± 1</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>Clorgyline, 1 μM (4)</td>
<td>7 ± 0</td>
<td>62 ± 1</td>
<td>41 ± 0</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>Clorgyline, 10 μM (4)</td>
<td>8 ± 0</td>
<td>61 ± 2</td>
<td>47 ± 1</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>

**Table 1: Preliminary experiments on effects of monoamine oxidase inhibitors on medium levels (pmol/well, means ± S.E.M.) of catechols after 180 minutes**

**Numbers of replicates are in parentheses.**
concentrations of DOPA changed biphassically in a mirrored image manner. For each of the three drugs, cell and medium concentrations of the deaminated metabolites decreased in a concentration-dependent manner (Tables 2 and 3). At the highest drug concentrations, cell and medium contents of DOPAL, Dopac, and Dopet were all significantly decreased compared with the lowest drug concentrations.

In the final experiment, where clorgyline at 1 nM (N = 6) was compared with rasagiline and selegiline at 500 nM (N = 6 each), all three drugs decreased DOPAL levels by more than 50% in both cell and medium (P < 0.0001 for each drug and matrix; Fig. 4). Clorgyline was more potent than the other two drugs in decreasing DOPAL levels, even at a 500-fold lower concentration (in cells, P = 0.002 compared with rasagiline, and P = 0.0003 compared with selegiline; in medium, P = 0.0005 compared with rasagiline, and P = 0.003 compared with selegiline). All three drugs also decreased cell and medium contents of Dopac, Dopet, and DHPG (P < 0.0001 for each drug and analyte), again with clorgyline being most potent.

At the effective drug concentrations, clorgyline, rasagiline, and selegiline increased DA contents in cells and medium (P < 0.0001 for each drug and analyte; Fig. 5). In increasing DA, clorgyline was more potent than the other two drugs (in cells, P = 0.0009 compared with rasagiline, and P < 0.0001.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DOPAL</th>
<th>Dopac</th>
<th>Dopet</th>
<th>DopA</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>1.00 ± 0.11 (23)</td>
<td>1.00 ± 0.07 (23)</td>
<td>1.00 ± 0.10 (23)</td>
<td>1.00 ± 0.09 (23)</td>
<td>1.00 ± 0.08 (23)</td>
</tr>
<tr>
<td>pmol/mL</td>
<td>1.80 ± 0.19</td>
<td>89.2 ± 6.3</td>
<td>10.2 ± 1.0</td>
<td>16.5 ± 1.5</td>
<td>1240 ± 94</td>
</tr>
</tbody>
</table>

**TABLE 2**

Effects of clorgyline, rasagiline, and selegiline on cell contents (fractions of control, means ± S.E.M.) of catechols after 180 minutes

Also displayed are the weighted mean values and weighted standard errors of the means (in pmol/mL) for zero-drug controls across the experiments. The control values in each experiment were assigned a value of 1.0. To calculate variability for 0 concentration controls, the S.E.M.s and the mean values across the experiments were weighted, and then the sum of the weighted S.E.M.s was divided by the sum of the weighted means.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DOPAL</th>
<th>Dopac</th>
<th>Dopet</th>
<th>DopA</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>1.00 ± 0.02 (3)</td>
<td>0.93 ± 0.06 (3)</td>
<td>0.96 ± 0.10 (3)</td>
<td>0.91 ± 0.26 (3)</td>
<td>1.05 ± 0.08 (3)</td>
</tr>
<tr>
<td>10</td>
<td>0.91 ± 0.19 (6)</td>
<td>0.82 ± 0.07 (6)</td>
<td>0.74 ± 0.08 (6)</td>
<td>0.90 ± 0.09 (6)</td>
<td>1.05 ± 0.03 (6)</td>
</tr>
<tr>
<td>100</td>
<td>0.65 ± 0.21 (6)</td>
<td>0.29 ± 0.10 (10)</td>
<td>0.45 ± 0.08 (10)</td>
<td>1.08 ± 0.22 (10)</td>
<td>1.17 ± 0.04 (10)</td>
</tr>
<tr>
<td>500</td>
<td>ND (6)**</td>
<td>0.13 ± 0.01 (6)</td>
<td>0.05 ± 0.01 (4)</td>
<td>0.97 ± 0.19 (6)</td>
<td>1.50 ± 0.11 (6)</td>
</tr>
<tr>
<td>1000</td>
<td>0.18 ± 0.12 (7)**</td>
<td>0.10 ± 0.01 (9)</td>
<td>0.24 ± 0.09 (8)</td>
<td>0.42 ± 0.06 (9)**</td>
<td>1.07 ± 0.10 (9)</td>
</tr>
<tr>
<td>10,000</td>
<td>0.19 ± 0.12 (7)**</td>
<td>ND (6)**</td>
<td>0.06 ± 0.01 (7)</td>
<td>0.15 ± 0.03 (5)</td>
<td>0.50 ± 0.08 (7)**</td>
</tr>
</tbody>
</table>

**TABLE 3**

Effects of clorgyline, rasagiline, and selegiline on medium contents (fractions of control, means ± S.E.M.) of catechols after 180 minutes

Data shown only for three or more replicates. Numbers of replicates are in parentheses. To calculate variability for 0 concentration controls, the S.E.M.s and the mean values across the experiments were weighted, and then the sum of the weighted S.E.M.s was divided by the sum of the weighted means.

<table>
<thead>
<tr>
<th>Drug</th>
<th>DOPAL</th>
<th>Dopac</th>
<th>Dopet</th>
<th>DopA</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>1.00 ± 0.11 (23)</td>
<td>1.00 ± 0.07 (23)</td>
<td>1.00 ± 0.10 (23)</td>
<td>1.00 ± 0.09 (23)</td>
<td>1.00 ± 0.08 (23)</td>
</tr>
<tr>
<td>pmol/mL</td>
<td>1.80 ± 0.19</td>
<td>89.2 ± 6.3</td>
<td>10.2 ± 1.0</td>
<td>16.5 ± 1.5</td>
<td>1240 ± 94</td>
</tr>
</tbody>
</table>

**TABLE 4**

Effects of clorgyline, rasagiline, and selegiline on medium contents (fractions of control, means ± S.E.M.) of catechols after 180 minutes

Data shown only for three or more replicates. Numbers of replicates are in parentheses. To calculate variability for 0 concentration controls, the S.E.M.s and the mean values across the experiments were weighted, and then the sum of the weighted S.E.M.s was divided by the sum of the weighted means.

IP, interfering peak; ND, not detected.

*Different from control by one-group t test, P < 0.05.

**P < 0.01.
compared with selegiline; in medium, \( P < 0.0001 \) each). The three drugs also decreased medium DOPA levels (\( P < 0.0001 \) for each drug); however, cell DOPA contents sporadically were high.

Clorgyline, rasagiline, and selegiline increased Cys-DA levels in cells and medium (Figs. 3 and 5). The increase in medium Cys-DA induced by clorgyline was larger than that by rasagiline or selegiline (\( P < 0.0001 \) for both comparisons); the increases in cell Cys-DA were similar for the three drugs.

**Effects of Rasagiline on Plasma Levels of Catechols.**

Demographic data for the clinical study are shown in Table 4. Patients on rasagiline had lower plasma mean levels of the deaminated metabolites DOPAC and DHPG than did healthy volunteers or patients not on rasagiline. Plasma DOPA was also lower in the rasagiline-treated group.

**Discussion**

The catecholaldehyde hypothesis predicts that decreasing production of the autotoxic DA metabolite DOPAL should slow the neurodegenerative process in Parkinson’s disease and related disorders. MAO inhibition would be a straightforward means to test this concept. In this study, we compared different MAO inhibitors in terms of the ability to decrease DOPAL production, and explored possible secondary effects that might augment or attenuate the beneficial effects of MAO inhibition.

**Clorgyline, Rasagiline, and Selegiline Decrease Endogenous DOPAL Production**

This study shows that the MAO-A inhibitor clorgyline and the MAO-B inhibitors rasagiline and selegiline all decrease endogenous production of DOPAL in PC12 cells. Consistent with the dominance of MAO-A in the enzymatic deamination of catecholamines in PC12 cells, clorgyline was far more potent than rasagiline or selegiline. A 50% reduction in DOPAL levels was attained at less than 1 nM clorgyline, and clorgyline at 1 nM was more potent than rasagiline or selegiline at 500 nM. Nevertheless, at 500 nM, rasagiline and selegiline decreased levels of DOPAL and other deaminated metabolites (**** \( P < 0.0001 \) compared with vehicle control). Clorgyline at 1 nM was more potent than rasagiline or selegiline at 500 nM. CON, control.

**Reversible MAO-A Inhibitors Are Relatively Ineffective in Decreasing DOPAL Production**

Reversible MAO-A inhibitors are less likely than irreversible MAO-A inhibitors to increase the risk of a cheese effect. In preliminary experiments, we evaluated three such drugs: toloxatone, moclobemide, and minaprine. All three were ineffective in decreasing endogenous DOPAL production at concentrations that could be attained clinically. Low efficacy in inhibiting intracellular MAO-A may provide an explanation...
for why these reversible MAO-A inhibitors produce little or no cheese effect.

**Indirect Effects of MAO Inhibition**

**Vesicular Uptake and Constitutive Release.** Clorgyline, rasagiline, and selegiline all increased contents of endogenous DA in the cells and medium. According to the concept depicted in Fig. 1, MAO inhibition, via buildup of cytoplasmic DA, augments vesicular uptake of DA and constitutive release of DA. Theoretically, this would be beneficial in Parkinson’s disease by increasing the delivery of DA to its receptors.

**Decreased Tyrosine Hydroxylase Activity.** Clorgyline, rasagiline, and selegiline concentration-dependently decreased endogenous DOPA production. It is known that manipulations that build up cytoplasmic DA attenuate tyrosine hydroxylase activity by feedback inhibition (Karobath, 1971; Carlsson et al., 1976; Ames et al., 1978; Wallace, 2007). Therefore, MAO inhibition, through increased cytoplasmic DA content, may decrease DOPA production by inhibition of tyrosine hydroxylase.

At high concentrations, clorgyline produced biphasic effects on medium concentrations of DA, NE, and Cys-DA and mirror-image biphasic effects on medium concentrations of DOPA (Fig. 3). A potential explanation for this unanticipated effect is that, at high concentrations, clorgyline may inhibit activity of l-aromatic-amino-acid decarboxylase. This would tend to decrease cytoplasmic DA levels and consequently decrease DA, NE, and Cys-DA levels in the medium while building up DOPA levels. Clorgyline has been reported to decrease activities of both tyrosine hydroxylase and l-aromatic-amino-acid decarboxylase in mouse striatum (Cho et al., 1996).
DA Auto-oxidation. MAO inhibition increases spontaneous auto-oxidation of cytoplasmic DA, as indicated by augmented Cys-DA content in the striatum of guinea pigs (Fornstedt and Carlsson, 1991). The present results confirm this effect, since clorglyline, rasagiline, and selegiline all increased endogenous Cys-DA production. Basic experiments have reported that both DA quinone and Cys-DA are toxic (Montine et al., 1997).

These findings may bear on the issue of possible neuroprotection by MAO inhibition. Larger multicenter clinical trials of MAO inhibitors in Parkinson’s disease have been disappointing (de la Fuente-Fernandez et al., 2010). Our data may shed light on the less-than-hoped-for results. Beneficial effects of decreasing DOPAL production might have been offset by deleterious effects of increased levels of dopamine auto-oxidation products.

Rasagiline and Plasma Neurochemical Indices of MAO-A Inhibition. The fact that dopaminergic neurons mainly express MAO-A raises the issue about whether doses of MAO-B inhibitors required to decrease DOPAL production can be attained clinically. The maximum plasma concentration for clinically used drug is about 60 nM for rasagiline (Thebault et al., 2004) and about 10 nM for selegiline (Lohle and Storch, 2008); however, plasma concentrations may not relate directly to the extent of MAO-A inhibition in catecholaminergic neurons. Selegiline delivered by patch or effervescent tablet (to avoid first-pass metabolism by the liver) decreases MAO-A activity in the human brain (Fowler et al., 2015), and at doses used clinically, selegiline also reduces plasma levels of the MAO-A metabolites DOPAC and DHPG (Eisenhofer et al., 1986). No previous study has reported on the effects of rasagiline on plasma levels of MAO-A metabolites at the clinically used dose of 1 mg per day. We therefore reviewed clinical neurochemical data on plasma levels of these catechols in parkinsonian patients. Patients on rasagiline had lower plasma DOPAC and DHPG levels compared to patients not on rasagiline and healthy controls, indicating decreased MAO-A activity in sympathetic nerves (Hovevey-Sion et al., 1989; Eisenhofer et al., 1996; Lenders et al., 1996). Plasma DOPA was also decreased, and in light of the present results about DOPA production in PC12 cells, reduced plasma DOPA in rasagiline-treated patients might reflect decreased tyrosine hydroxylase activity in sympathetic nerves (Kvetansky et al., 1992).

Our experiments focused on dopaminergic cells and, based on the catecholaldehyde hypothesis, a pharmacological intervention that might slow dopaminergic neuronal cell death. There is a range of pathologic abnormalities in Parkinson’s disease (McCann et al., 2015), and thus the issue of why neurons in other neurotransmitter systems (e.g., noradrenergic, serotonergic, cholinergic) are also lost in Parkinson’s disease is left open. The aldehyde hypothesis might extend to noradrenergic and serotonergic neurons, because in these neurons, a buildup of neurotransmitter in the cytoplasm would be expected to generate cytotoxic aldehydes via MAO, as in dopaminergic neurons. Less clear is whether the present results are relevant to mechanisms of loss of cholinergic neurons, such as in the dorsal motor nucleus of the vagus nerve.

In summary, the present results indicate that clorglyline is extremely potent at decreasing endogenous DOPAL production. The MAO-B inhibitors rasagiline and selegiline, at much higher but clinically attainable concentrations, also decrease DOPAL production, whereas reversible MAO-A inhibitors are relatively ineffective. The pathogenetic meanings of both enzymatic and spontaneous oxidation of DA are unknown. Clinical neuroprotection trials in patients with Parkinson’s disease could address this issue. Based on the present results, however, one should consider secondary effects of MAO inhibition—especially increased spontaneous oxidation of DA.

Authorship Contributions
Participated in research design: Goldstein, Jinsmaa, Kopin, Sharrahi.
Conducted experiments: Jinsmaa, Sullivan.
Contributed new reagents or analytic tools: Holmes.
Performed data analysis: Goldstein, Jinsmaa, Sullivan, Sharrahi
Wrote or contributed to the writing of the manuscript: Goldstein, Jinsmaa, Kopin, Sharrahi.

References


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