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Importance of monoamine oxidase A in the bioactivation of neurotoxic analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

RICHARD E. HEIKKILA*, M. VICTORIA KINDT*†, PATRICIA K. SONSALLA*, ANDREW GIOVANNI*, STEPHEN K. YOUNGSTER*, KATHLEEN A. MCKEOWN‡§, AND THOMAS P. SINGER‡§

*Department of Neurology, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ 08854; ‡Molecular Biology Division, Veterans Administration Medical Center, San Francisco, CA 94121; and §Department of Biochemistry-Biophysics and Pharmacy, University of California, San Francisco, CA 94143

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ABSTRACT **1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent dopaminergic neurotoxin that causes biochemical, pharmacological, and pathological deficits in experimental animals similar to those seen in human parkinsonian patients. All of the deficits can be prevented by treating mice with selective inhibitors of monoamine oxidase B (MAO-B), including deprenyl, prior to MPTP administration. We now report that the dopaminergic neurotoxicity of two potent MPTP analogs, namely the 2′-methyl and 2′-ethyl derivatives (2′-MeMPTP and 2′-EtMPTP), cannot be prevented by deprenyl pretreatment. However, the neurotoxicity of these two analogs can be prevented by pretreatment with a combination of deprenyl and the selective MAO-A inhibitor clorgyline at doses that are sufficient to almost completely inhibit both MAO-B and MAO-A activities. Moreover, the neurotoxicity of 2′-EtMPTP (but not of 2′-MeMPTP and MPTP) can be significantly attenuated by clorgyline alone. There was a parallel between the capacity of the MAO inhibitors to decrease the brain content of the pyridinium species after administration of the tetrahydropyridines and the capacity of the MAO inhibitors to protect against the neurotoxic action of the tetrahydropyridines. The data support the conclusion that both 2′-MeMPTP and 2′-EtMPTP are bioactivated to pyridinium species to a significant extent by MAO-A. Further, it appears that the formation of the pyridinium species plays an important role in the neurotoxic process.**

The inadvertent self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by several young drug abusers resulted in a severe parkinsonian syndrome (1–3). Moreover, the administration of MPTP to nonhuman primates causes behavioral, biochemical, and pathological deficits very similar to those observed in patients with idiopathic Parkinson disease (4, 5). There are at least two necessary steps involved in the MPTP-induced destruction of nigrostriatal dopaminergic neurons. First, MPTP is oxidized by monoamine oxidase B (MAO-B) to the 1-methyl-4-phenyl-2,3-dihydropyridinium species ($MPDP^+$), which is then transformed either spontaneously or enzymatically to the 1-methyl-4-phenylpyridinium species (MPP^+), the primary metabolite of MPTP found in the brains of experimental animals (6–9). Second, MPP^+ is actively transported into the dopaminergic neurons by the dopamine-uptake system (10, 11). The importance of these two steps is indicated by the observations that pretreatment of experimental animals with inhibitors of either MAO-B (12–15) or dopamine uptake (10, 16, 17) prevents MPTP-induced neurotoxicity.

We recently found that a 2′-substituted analog of MPTP, 1-methyl-4-(2′-methylphenyl)-1,2,3,6-tetrahydropyridine (2′-MeMPTP) (18, 19) was a more potent nigrostriatal dopaminergic neurotoxin than MPTP in mice. As with MPTP, pretreatment with inhibitors of dopamine uptake prevented 2′-MeMPTP-induced neurotoxicity (19). However, unlike the case with MPTP, pretreatment with low and selective doses of MAO-B inhibitors did not prevent 2′-MeMPTP-induced neurotoxicity (19). We now report that in order to prevent the nigrostriatal dopaminergic neurotoxicity of 2′-MeMPTP in mice, it is necessary to inactivate both MAO-B and MAO-A almost completely. We also report results of metabolic studies with 2′-MeMPTP and metabolic and neurotoxicity studies with another structural analog, 1-methyl-4-(2′-ethylphenyl)-1,2,3,6-tetrahydropyridine (2′-EtMPTP), in mice *in vivo*. The results support the conclusion that both of these 2′-substituted analogs of MPTP are bioactivated to pyridinium species to a significant extent by MAO-A.

MATERIALS AND METHODS

Sources of Compounds. 2′-MeMPTP hydrochloride, 2′-EtMPTP hydrochloride, 2′-$MeMPDP^+$ perchlorate, 2′-$EtMPDP^+$ perchlorate, 2′-$MeMPP^+$ iodide, and 2′-$EtMPP^+$ iodide were synthesized in our laboratory; their structures and purities were corroborated by nuclear magnetic resonance and mass spectral analyses. MPTP hydrochloride, $MPDP^+$ perchlorate, MPP^+ iodide, and clorgyline hydrochloride were purchased from Research Biochemicals (Natick, MA). 1-Deprenyl hydrochloride was a gift from J. Knoll (Semmelweis University, Budapest, Hungary).

Metabolism of MPTP, 2′-MeMPTP, and 2′-EtMPTP in Mice. Male Swiss-Webster mice (25 g; Taconic Farms, Germantown, NY) received a single subcutaneous injection of MPTP (40 mg/kg), 2′-MeMPTP (7.5 mg/kg), or 2′-EtMPTP (15 mg/kg); doses are in mg of free base per kg of body weight. These doses were chosen because they produced a similar depletion of neostriatal dopamine content. In other experiments, mice received 0.116 mmol of each compound per kg. This dose is equivalent to 20 mg of MPTP per kg. Pretreatment with the MAO inhibitors consisted of intraperitoneal injections of deprenyl hydrochloride (2.5 mg/kg), clorgyline hydrochloride (2.5 mg/kg), or a combination of the two MAO inhibitors (each at 2.5 mg/kg) administered 1 and 2 days prior to 2′-MeMPTP or 2′-EtMPTP administration; other mice received no pretreatment. Deprenyl treatment caused an ≈90% decrement in neostriatal MAO-B activity and no decrease in MAO-A activity; clorgyline treatment produced an ≈90% decrement in neostriatal MAO-A activity and no significant effect on MAO-B activity.

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Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; MPP^+, 1-methyl-4-phenylpyridinium; 2′-MeMPTP, 1-methyl-4-(2′-methylphenyl)-1,2,3,6-tetrahydropyridine; 2′-EtMPTP, 1-methyl-4-(2′-ethylphenyl)-1,2,3,6-tetrahydropyridine; MAO, monoamine oxidase.

†Present address: Department of Safety Assessment, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

In mice treated with a combination of deprenyl and clorgyline, both neostriatal MAO-A and MAO-B activity were decreased by about 90% (data not shown). At the indicated times after MPTP, 2'-MeMPTP, or 2'-EtMPTP administration, the mice were stunned by a blow to the head and decapitated. The brains were rapidly removed and bisected in the sagittal plane. The right hemisphere of the brain was immediately homogenized in 5 volumes of 0.2 M perchloric acid. The homogenate was centrifuged and the compounds in the resulting supernatant were quantified by HPLC with UV detection, according to a modification of the procedure of Shinka *et al.* (20). The chromatograph was equipped with a cation-exchange precolumn and analytical column (4.6 mm × 25 cm; Altex, Ultrasil-Cx, 10-μm particle size). The mobile phase consisted of 0.05 M acetic acid/0.0375 M diethylamine (pH 6.0), and acetonitrile (82:18, vol/vol). The compounds were monitored at the following wavelengths: MPTP, 245 nm; $MPDP^+$, 2'-$MeMPDP^+$, and 2'-$EtMPDP^+$, 345 nm; MPP^+, 295 nm; 2'-Me- and 2'-EtMPTP, 228 nm; and 2'-Me- and 2'-$EtMPP^+$, 285 nm. The limits of detection of each of the tetrahydropyridines, the dihydropyridiniums, and the pyridiniums were similar to those described by Shinka *et al.* (20) for MPTP, $MPDP^+$, and MPP^+, respectively. Quantitation was made by comparison of peak height ratios in the samples with those of standards, and corrections were made for recovery.

2'-MeMPTP and 2'-EtMPTP Neurotoxicity in Mice. Mice were injected as described for the metabolic experiments (see doses in Table 2). Three weeks later they were decapitated and the neostriatal content of dopamine and its metabolites was determined by HPLC with electrochemical detection (19, 21).

Purification of MAO-A and MAO-B. MAO-B was purified from bovine liver exactly as described (22). MAO-A was purified from human placenta as described (23) except for minor modifications.

Measurement of Activity of Purified MAO-A and MAO-B *in Vitro*. The oxidation of the tetrahydropyridine analogs by MAO-A and MAO-B was followed polarographically with a Clark oxygen electrode. The 1.5-ml reaction mixture, containing 50 mM sodium phosphate (pH 7.2), 0.2% (wt/vol) Triton X-100, and 0.1–1.25 mM substrate, was equilibrated to 30°C. The reaction was initiated with 50 μg of MAO-A or 35 μg of MAO-B. Activities were calculated from initial linear rates of oxygen consumption. Turnover numbers were calculated per mol of covalently bound flavin.

Measurement of MAO Activity in Brain Homogenates. A crude mitochondrial preparation was made from whole brains from male Swiss-Webster mice (Taconic Farms) as described (18, 24). In this strain of mice, whole-brain levels of MAO-A and MAO-B are relatively similar (unpublished observations). Aliquots of the tissue preparation corresponding to 30 mg of tissue (original wet weight) were used for the MAO assay. MAO-A activity was defined as activity in the presence of 0.25 μM deprenyl, and MAO-B activity was defined as activity in the presence of 0.25 μM clorgyline. These concentrations of the MAO inhibitors are selective and complete for the appropriate form of MAO (24).

RESULTS

A time course for brain levels of MPP^+, 2'-$MeMPP^+$, and 2'-$EtMPP^+$ and their corresponding tetrahydropyridines after an injection of equivalent amounts of the tetrahydropyridines is presented in Fig. 1. The brain levels of 2'-$MeMPP^+$ are greater over the time course than the levels of 2'-$EtMPP^+$, which in turn are greater than the levels of MPP^+. This difference in brain levels of the pyridiniums parallels the potency of their corresponding tetrahydropyridines as neurotoxins (2'-MeMPTP > 2'-EtMPTP > MPTP). Brain levels of MPP^+, 2'-$MeMPP^+$, and 2'-$EtMPP^+$ at 1 hr after injection of various amounts of the appropriate tetrahydropyridine are shown in Table 1. These doses were determined in prelimi-

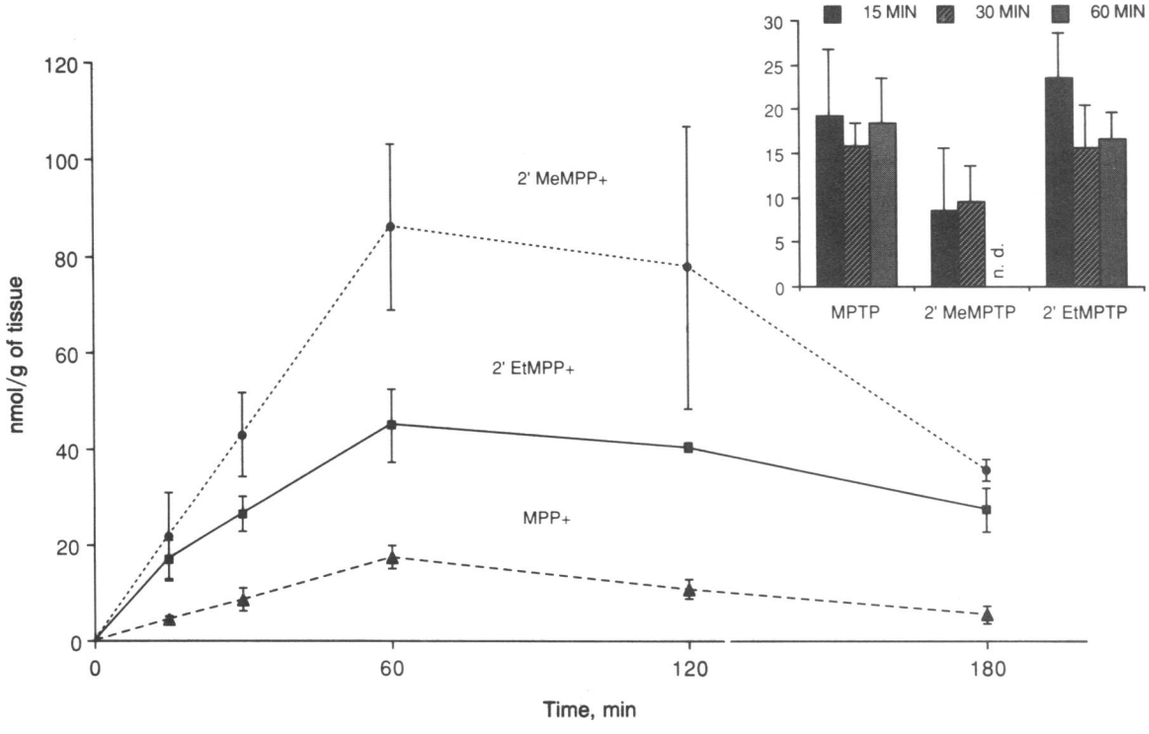

FIG. 1. **Brain levels of tetrahydropyridines (*Inset*) and pyridiniums after subcutaneous injection of an equivalent dose of MPTP, 2'-MeMPTP, or 2'-EtMPTP (0.116 mmol/kg). Data are the mean brain content (nmol/g of tissue) ± SD for 4 mice per compound per time point. There was no tetrahydropyridine detectable at the 2-hr and 3-hr time points, and 2'-MeMPTP was not detectable (n.d.) at 1 hr. Where not shown, the error bar is within the size of the symbol.**

Table 1. Tetrahydropyridine and pyridinium species in whole brain 1 hr after subcutaneous injection of MPTP, 2′-MeMPTP, or 2′-EtMPTP

Neurotoxin injected	Pretreatment	nmol/g of tissue	
		Tetrahydropyridine*	Pyridinium
MPTP (40 mg/kg)	None	63.5 ± 12.5	27.9 ± 7.8
	Deprenyl	72.5 ± 24.8	9.0 ± 0.3†
	Clorgyline	59.7 ± 6.0	29.4 ± 2.7
	Deprenyl/clorgyline	60.6 ± 2.3	<3.2†‡
2′-MeMPTP (7.5 mg/kg)	None	ND	20.2 ± 1.6
	Deprenyl	ND	17.6 ± 1.9
	Clorgyline	ND	14.9 ± 1.7†
	Deprenyl/clorgyline	ND	6.7 ± 0.2†
2′-EtMPTP (15 mg/kg)	None	ND	33.2 ± 2.4
	Deprenyl	ND	31.7 ± 10.2
	Clorgyline	ND	17.3 ± 2.2†
	Deprenyl/clorgyline	ND	<3.2†‡

There were three mice per group; data represent the mean content (nmol/g of tissue wet weight) ± SD. The dihydropyridinium species for all three tetrahydropyridine analogs was present at earlier time periods, but very little or none was detectable at 1 hr.
*ND, the tetrahydropyridine was not detectable 1 hr after injection although it was at earlier time points.
†$P < 0.05$ compared to control.
‡Limit of detection.

nary experiments to produce a comparable degree of nigrostriatal dopaminergic neurotoxicity. A dose of deprenyl selective for inhibiting MAO-B (2.5 mg/kg) reduced the MPP^+ concentration from 27.9 ± 7.8 to 9.0 ± 0.3 nmol/g of tissue (68% decrease). A dose of clorgyline selective for inhibiting MAO-A (also 2.5 mg/kg) had no significant effect on the brain concentration of MPP^+. These data are in accord with the observations that a selective dose of deprenyl protects against MPTP-induced dopaminergic neurotoxicity and inhibits MPP^+ formation, whereas a selective dose of clorgyline does not protect and has no effect on MPP^+ formation (9, 12, 13). Combined clorgyline and deprenyl pretreatment also substantially reduced MPP^+ formation (>89% decrease).

The formation of 2′-$MeMPP^+$ from 2′-MeMPTP was not significantly inhibited in mice pretreated with deprenyl alone but was significantly attenuated (26%) by clorgyline pretreatment (Table 1). However, combined deprenyl and clorgyline pretreatment reduced the concentration of 2′-$MeMPP^+$ to 6.7 ± 0.2 compared to a control level of 20.2 ± 1.6 nmol/g of tissue (67% decrease). In parallel experiments, pretreatment of mice with the combination of deprenyl and clorgyline protected against 2′-MeMPTP-induced neurotoxicity, whereas each inhibitor alone was ineffective (Table 2).

Pretreatment with clorgyline alone reduced the formation of 2′-$EtMPP^+$ from 2′-EtMPTP by 48%. In contrast, treatment with deprenyl alone had no significant effect. Pretreatment with a combination of deprenyl and clorgyline resulted in the largest reduction in the brain 2′-$EtMPP^+$ concentration. In separate experiments (Table 2) clorgyline alone, but not deprenyl alone, and the combination of deprenyl and clorgyline provided substantial protection against 2′-EtMPTP-induced toxicity. There is thus a clear relationship between the capacity of MAO inhibitors to prevent pyridinium formation *in vivo* (Table 1) and their capacity to protect against 2′-MeMPTP- and 2′-EtMPTP-induced neurotoxicity (Table 2).

Kinetic constants for the oxidation of MPTP and its analogs by pure MAO-A and MAO-B are shown in Table 3. Based on the K_m values, MPTP and its analogs are good substrates for pure MAO-A and comparable to a routinely used MAO-A substrate, kynuramine. Based on turnover numbers, 2′-MeMPTP is better than 2′-EtMPTP, which in turn is much better than MPTP; none had a turnover number as high as kynuramine. The K_m values for the three tetrahydropyridines for MAO-B are comparable, and similar to that of the MAO-B substrate benzylamine, and in each case

Table 2. Effects of deprenyl and/or clorgyline pretreatment on the neurotoxicity of 2′-MeMPTP and 2′-EtMPTP

Neurotoxin	Pretreatment	*n*	Neostriatal dopamine, μg/g of tissue
None	None	4	12.6 ± 1.8
	Deprenyl	4	11.0 ± 1.6
	Clorgyline	4	11.8 ± 1.6
	Deprenyl/clorgyline	4	12.7 ± 0.6
2′-MeMPTP	None	8	6.2 ± 0.8*
	Deprenyl	8	7.1 ± 1.1*
	Clorgyline	8	8.8 ± 1.8*
	Deprenyl/clorgyline	8	12.0 ± 1.7†
2′-EtMPTP	None	8	4.8 ± 1.5*
	Deprenyl	8	7.2 ± 3.1*
	Clorgyline	8	10.6 ± 1.0†
	Deprenyl/clorgyline	8	13.0 ± 1.1†

Male Swiss-Webster mice were pretreated with the appropriate MAO inhibitor(s) at 2.5 mg/kg on each of 2 successive days. The next day some of the mice received one subcutaneous injection of 2′-MeMPTP (7.5 mg/kg) or 2-′EtMPTP (15 mg/kg). The mice were killed 3 weeks later and the dopamine content was determined. Data are the mean neostriatal dopamine content ± SD.
*$P < 0.05$ compared to control.
†$P < 0.05$ compared to 2′-MeMPTP or 2′-EtMPTP alone.

Table 3. Kinetic constants for oxidation of MPTP, 2′-MeMPTP, and 2′-EtMPTP by purified MAO-A (human placenta) and purified MAO-B (bovine liver): a comparison with known substrates

Compound	MAO	K_m, μM	Turnover number, nmol per min per mol of flavin
Kynuramine	A	120	154
Benzylamine	B	390	415
MPTP	A	140	20
	B	390	204
2′-MeMPTP	A	140	83
	B	280	357
2′-EtMPTP	A	77	53
	B	770	227

Experiments were done polarographically at 30°C. Data are the mean values for triplicate determinations.

Table 4. Rates of oxidation of MPTP, 2'-MeMPTP, and 2'-EtMPTP in mitochondrial preparations from the whole brains of Swiss-Webster mice

Compound	MAO	Apparent K_m, μM	Apparent V_{max}, nmol of H_2O_2 per hr per g of tissue
MPTP	A	ND	ND
	B	84 ± 36	895 ± 136
2'-MeMPTP	A	27 ± 2	509 ± 44
	B	53 ± 12	2691 ± 150
2'-EtMPTP	A	40 ± 6	875 ± 102
	B	125 ± 43	1127 ± 139

MAO-A activity was determined in the presence of 0.25 μM deprenyl, and MAO-B activity was determined in the presence of 0.25 μM clorgyline. Data are the mean ± SD for 3–5 separate determinations. ND, not determined (the activity for MPTP was too low to determine with the method utilized).

higher than the corresponding value for MAO-A. The turnover numbers for all three compounds for MAO-B are quite high and are comparable to or less than that for benzylamine. Most of these findings were confirmed in mitochondrial preparations from whole brain (Table 4).

As is apparent from the kinetic data presented in Table 4, there was an increase in the oxidation rate with an increasing concentration of the tetrahydropyridines by both MAO-A and MAO-B in the whole brain mitochondrial preparations (see Fig. 2 for representative data with 2'-EtMPTP). However, the proportion of the total oxidation carried out by MAO-A decreased with increasing concentration, while the proportion carried out by MAO-B increased. For example, at 10 μM 2'-EtMPTP, 73% of the total oxidation was mediated by MAO-A (27% by MAO-B). At 100 μM and 1000 μM, the proportion mediated by MAO-A was 55% and 45%, respectively.

DISCUSSION

Several groups of investigators have reported that MPTP-induced dopaminergic neurotoxicity can be prevented in experimental animals by inhibitors of dopamine uptake (10, 16). In previous studies, we have similarly found that inhibitors of dopamine uptake prevent both 2'-MeMPTP- (19) and 2'-EtMPTP-induced (unpublished observations) nigrostriatal toxicity *in vivo* in mice. Furthermore, MPP^+, 2'-$MeMPP^+$, and 2'-$EtMPP^+$, but not their corresponding tetrahydropyridines, are good substrates for the dopamine-transport system *in vitro* (refs. 10 and 16; unpublished observations). These observations suggest that most of the bioactivation of MPTP, 2'-MeMPTP, and 2'-EtMPTP to the corresponding

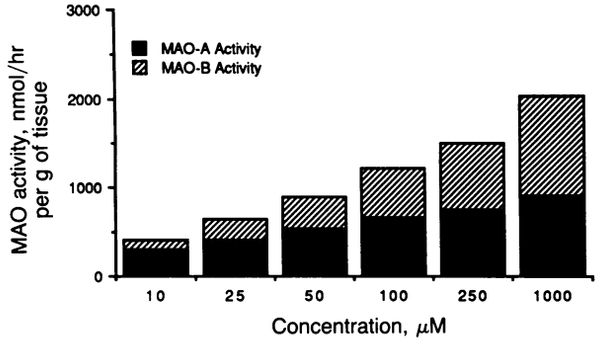

FIG. 2. Dose–response curve for the oxidation of 2'-EtMPTP in mitochondrial preparations from whole brains of Swiss-Webster mice. Data are taken from an individual experiment similar to the kinetic experiments presented in Table 4. Almost identical data were obtained in each of the other experiments.

pyridinium species does not take place within the dopaminergic neuron, which in mice is known to contain predominantly MAO-A and little or no MAO-B. After their formation, the pyridinium species would be concentrated within the dopaminergic neuron by the dopamine-transport system. The pyridinium forms of 2'-MeMPTP and 2'-EtMPTP are, like MPP^+, good inhibitors of complex I mitochondrial respiration (refs. 18, 25, and 26; unpublished observations). The greater nigrostriatal dopaminergic neurotoxicity of 2'-MeMPTP and 2'-EtMPTP compared to MPTP in the mouse appears to be due, at least in part, to two phenomena. First, each of these analogs is a better substrate for MAO than is MPTP. Second, after an equivalent dose of the neurotoxins, there are greater brain concentrations of 2'-$MeMPP^+$ and 2'-$EtMPP^+$ and longer biological half-lives compared to MPP^+ (Fig. 1).

Selective inhibitors of MAO-B are able to protect against MPTP-induced nigrostriatal dopaminergic neurotoxicity. This is most likely due to the ability of the MAO-B inhibitors to prevent the formation of MPP^+ from MPTP (9, 12–15, 27). The inability to protect against 2'-MeMPTP- and 2'-EtMPTP-induced nigrostriatal neurotoxicity with selective inhibitors of MAO-B appears to relate to the inability of these inhibitors to diminish the formation of the pyridinium ions. Only when both MAO-B and MAO-A are inhibited, by the combination of deprenyl and clorgyline, does the brain concentration of 2'-$MeMPP^+$ decrease substantially (Table 1) and is there protection against 2'-MeMPTP-induced neurotoxicity (Table 2). We have found that MDL 72145, AGN-1133, and pargyline, at doses capable of inhibiting both MAO-A and MAO-B activity by >90%, protect against 2'-MeMPTP-induced neurotoxicity (19, 28). In the case of 2'-EtMPTP, inhibition of MAO-A alone is sufficient to attenuate neurotoxicity (Table 2) and to reduce the concentration of 2'-$EtMPP^+$ in the brain substantially (Table 1). Moreover, the combination of deprenyl and clorgyline is very effective in decreasing 2'-$EtMPP^+$ formation (Table 1) and also protects fully against 2'-EtMPTP-induced neurotoxicity (Table 2).

Based on turnover numbers or apparent V_{max} values (Tables 3 and 4), MPTP, 2'-MeMPTP, and 2'-EtMPTP are better substrates for MAO-B than for MAO-A. In contrast, based on K_m values, all appear to be better substrates for MAO-A than for MAO-B. Because of its relatively low turnover number for MAO-A compared to MAO-B, most MPTP oxidation *in vitro* is carried out by MAO-B; moreover, MPTP appears to be bioactivated primarily by MAO-B *in vivo* (e.g., see effect of deprenyl but no effect of clorgyline, Table 1). Based on turnover numbers or apparent V_{max} values (Tables 3 and 4), both 2'-MeMPTP and 2'-EtMPTP are better substrates for MAO-A than is MPTP. However, based on K_m values, it is apparent that 2'-MeMPTP and 2'-EtMPTP (and even MPTP) are quite good substrates for MAO-A. If one takes into account these low K_m values for oxidation by MAO-A compared to MAO-B, combined with the appropriate V_{max} values, it is clear that at low substrate concentrations a significant proportion of the total oxidation of the 2'-substituted tetrahydropyridines can be carried out *in vitro* in brain preparations by MAO-A. As an example, at 10 μM 2'-EtMPTP, 73% of the total oxidation was mediated by MAO-A and only 27% by MAO-B (Fig. 2). This proportion of 2'-EtMPTP oxidation carried out by MAO-A decreased with increasing concentrations, although even at 1000 μM, a significant proportion (45%) of 2'-EtMPTP oxidation was mediated by MAO-A.

If the brain concentration of the tetrahydropyridine were quite low (i.e., well below the appropriate K_m values for MAO-B and at or below the K_m values for MAO-A), it is obvious that a significant proportion of the oxidation and bioactivation of both MPTP analogs, and perhaps even of MPTP itself, could be mediated by MAO-A. In these exper-

iments, we measured the brain tetrahydropyridine concentrations in mice receiving MPTP, 2'-MeMPTP, or 2'-EtMPTP over a 3-hr period. The concentrations were well under 50 μM for most of the 3-hr period, which is theoretically in the range that MAO-A-catalyzed oxidation should take place to a significant extent (see K_m values in Table 4 and data of Fig. 2). That oxidation by MAO-A is relevant, at least with 2'-EtMPTP and perhaps with 2'-MeMPTP, is suggested by the data in Table 1 on the inhibition of pyridinium formation by clorgyline alone and the even more extensive inhibition of pyridinium formation by the combination of deprenyl and clorgyline than by deprenyl alone. This concept is reinforced by the observations that clorgyline alone and the combination of deprenyl and clorgyline protect against 2'-EtMPTP-induced neurotoxicity and that the combination of deprenyl and clorgyline protects against 2'-MeMPTP-induced neurotoxicity (Table 2). The greater effect of the deprenyl/clorgyline combination than of deprenyl alone on MPP^+ formation from MPTP (Table 1) is consistent with the premise that some MPTP may also be bioactivated by MAO-A.

There is an interesting structure–activity relationship that exists for this homologous series of compounds. Going from the 2'-H in MPTP to either the 2'-Me or the 2'-Et results in compounds that are considerably better than MPTP as substrates for MAO-A (i.e., higher turnover number in Table 3). This 2' position is obviously an important site for the interaction of this class of molecules with MAO-A. Perhaps this is due to a hydrophobic interaction between the substrate and the enzyme that results in conditions more favorable for oxidation to take place. That this 2' position is also important for MAO-B is exemplified by the observation that 2'-MeMPTP is such a good substrate for MAO-B. We have found other 2'-substituted analogs of MPTP (e.g., 2'-chloro) and also a few 3'-substituted analogs (e.g., 3'-chloro) that are good substrates for both MAO-A and MAO-B *in vitro* and that are bioactivated by MAO-A and/or MAO-B *in vivo* (data not shown).

The discovery that a systemically administered compound like MPTP could produce neuropathological and behavioral effects so similar to those produced in idiopathic parkinsonism has led to considerable speculation that a compound similar to MPTP may be involved in the pathogenesis of Parkinson disease (29). The finding that MAO-B plays an important role in MPTP-induced neurotoxicity has led to suggestions that MAO-B might play a role in the pathogenesis of parkinsonism. Were a 2'-MeMPTP- or 2'-EtMPTP-like molecule involved in the pathogenesis of parkinsonism, it follows that not only MAO-B, but also MAO-A, might play an important role in the development of the disease state. It is clear that investigators should not focus too intently on the premise that MPTP is the only tetrahydropyridine that might theoretically be responsible for causing idiopathic parkinsonism.

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1. Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M. & Kopin, I. J. (1979) *Psychiatry Res.* **1**, 249–254.
2. Langston, J. W., Ballard, P., Tetrud, J. W. & Irwin, I. (1983) *Science* **219**, 979–980.
3. Burns, R. S., Markey, S. P., Phillips, J. M. & Chiueh, C. C. (1984) *Can. J. Neurol. Sci.* **11**, 166–168.
4. Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M. & Kopin, I. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4546–4550.
5. Langston, J. W., Forno, L. S., Rebert, C. S. & Irwin, I. (1984) *Brain Res.* **292**, 390–394.
6. Chiba, K., Trevor, A. & Castagnoli, N., Jr. (1984) *Biochem. Biophys. Res. Commun.* **120**, 547–578.
7. Chiba, K., Peterson, L. A., Castagnoli, A., Trevor, A. J. & Castagnoli, N., Jr. (1985) *Drug Metab. Dispos.* **13**, 342–347.
8. Salach, J. I., Singer, T. P., Castagnoli, N., Jr., & Trevor, A. J. (1984) *Biochem. Biophys. Res. Commun.* **125**, 831–835.
9. Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S. & Herkenham, M. A. (1984) *Nature (London)* **311**, 464–467.
10. Javitch, J. A., D'Amato, R. J., Strittmatter, S. M. & Snyder, S. H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2173–2177.
11. Chiba, K., Trevor, A. & Castagnoli, N., Jr. (1985) *Biochem. Biophys. Res. Commun.* **128**, 1228–1232.
12. Heikkila, R. E., Manzino, L., Cabbat, F. S. & Duvoisin, R. C. (1984) *Nature (London)* **311**, 467–469.
13. Langston, J. W., Irwin, I., Langston, E. B. & Forno, L. S. (1984) *Science* **225**, 1480–1482.
14. Fuller, R. W. & Hemrick-Luecke, S. K. (1986) *Res. Commun. Chem. Pathol. Pharmacol.* **53**, 167–172.
15. Sundstrom, E. & Jonsson, G. (1985) *Eur. J. Pharmacol.* **110**, 293–299.
16. Mayer, R. A., Kindt, M. V. & Heikkila, R. E. (1986) *J. Neurochem.* **47**, 1073–1079.
17. Ricaurte, G. A., Langston, J. W., DeLanney, L. E., Irwin, I. & Brooks, J. D. (1985) *Neurosci. Lett.* **59**, 259–264.
18. Youngster, S. K., Sonsalla, P. K. & Heikkila, R. E. (1987) *J. Neurochem.* **48**, 929–934.
19. Sonsalla, P. K., Youngster, S. K., Kindt, M. V. & Heikkila, R. E. (1987) *J. Pharmacol. Exp. Ther.* **242**, 850–857.
20. Shinka, T., Castagnoli, N., Jr., Wu, E. Y., Hoag, M. K. P. & Trevor, A. J. (1987) *J. Chromatogr.* **398**, 279–287.
21. Sonsalla, P. K. & Heikkila, R. E. (1986) *Eur. J. Pharmacol.* **129**, 339–345.
22. Weyler, W. & Salach, J. I. (1981) *Arch. Biochem. Biophys.* **212**, 147–153.
23. Weyler, W. & Salach, J. I. (1985) *J. Biol. Chem.* **260**, 13,199–13,207.
24. Heikkila, R. E., Youngster, S. K., Manzino, L., Cabbat, F. S. & Duvoisin, R. C. (1985) *J. Neurochem.* **44**, 310–313.
25. Nicklas, W. J., Youngster, S. K., Kindt, M. V. & Heikkila, R. E. (1987) *Life Sci.* **40**, 721–729.
26. Ramsay, R. R., Salach, J. I. & Singer, T. P. (1986) *Biochem. Biophys. Res. Commun.* **134**, 743–748.
27. Jonsson, G., Sundstrom, E., Nwanze, E., Hallman, H. & Luthman, J. (1986) in *MPTP: A Neurotoxin Producing a Parkinsonian Syndrome*, eds. Markey, S. P., Castagnoli, N., Jr., Trevor, A. J. & Kopin, I. J. (Academic, New York), pp. 253–272.
28. Heikkila, R. E. & Sonsalla, P. K. (1987) *Can. J. Neurol. Sci.* **14**, 436–440.
29. Snyder, S. H. & D'Amato, R. J. (1986) *Neurology* **36**, 250–258.