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METABOLISM OF THE NEUROTOXIC TERTIARY AMINE, MPTP, BY BRAIN MONOAMINE OXIDASE

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SUMMARY. The neurotoxic chemical MPTP (1-methyl-4-phenyl-1,2,4,5-tetrahydropyridine) is metabolized by rat brain mitochrondrial fractions at a rate of 0.91 ± 0.02 nmoles/mg protein/min. The major metabolite has been identified as the 1-methyl-4-phenylpyridinium species. This biotransformation process is blocked by 10^{-7} M deprenyl and pargyline. MPTP itself inhibited the metabolism of benzylamine by brain mitochondrial fractions. These results are discussed in terms of possible bioactivation mechanisms that may be associated with the neurodegenerative properties of MPTP.

INTRODUCTION. MPTP (1-methyl-4-phenyl-1,2,4,5-tetrahydropyridine, 1, Figure 2) is a neurotoxic cyclic tertiary allylamine that has caused Parkinsonism in individuals exposed to the substance through self-administration (1,2). In laboratory animals MPTP causes the selective destruction of cells in the nigrostriatal tract of the CNS (3) by an unknown mechanism. By analogy with 6-hydroxydopamine (4), the neurodegenerative properties of MPTP might be mediated by biochemical lesions initiated through covalent modification of neuronal macromolecules. Although MPTP itself would be expected to display low inherent chemical reactivity, it may be biotransformed to reactive electrophilic and hence potentially cytotoxic intermediates by tissue enzymes known to metabolize tertiary amines, including the cytochrome P-450 mono-oxygenases (5,6) and the monoamine oxidases (7,8).

Mammalian brain tissues exhibit only low enzymic activities associated with cytochrome P-450 catalyzed reactions (9) but have high MAO activity (10). To investigate the possibility that MPTP is biotransformed by brain MAO to potentilly reactive neurotoxic species, we have examined the metabolism of MPTP in tissue homogenates and subcellular fractions derived from rat brain. A

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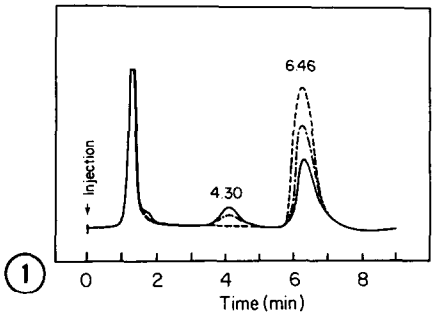
major metabolite of MPTP has been isolated from brain mitochondrial fraction incubation mixtures and its molecular structure has been characterized by chemical ionization (CI) mass and ^{1}H NMR spectral analyses and confirmed by comparisons with authentic synthetic material. Additionally, the effects of MAO inhibitors on this biotransformation process have been examined.

METERIALS AND METHODS. Benzylamine, pargyline and MPTP were obtained from the Aldrich Chemical Co., Milwaukee, WIS., U.S.A. Deprenyl and clorgyline were generously supplied by Dr. Thomas Singer, University of California, San Francisco, CA, U.S.A. and 1-methyl-4-phenylpyridinium iodide was a gift from Dr. Sanford Markey, National Institute of Mental Health, Bethesda, MD, U.S.A. All other chemicals were of reagent or, in the case of solvents, HPLC grade.

Male Wistar rats (150-200 g) were decapitated and whole brains removed and homogenized in 0.32 M sucrose to make a 10% homogenate. This was centrifuged to separate nuclear, mitochondrial, microsomal and soluble (cytoplasmic) subfractions (11). Protein concentrations were determined by the method of Lowry et al. (12). For analytical studies, incubations of MPTP (0.1 mM) with brain tissue fractions (1.5 or 2.0 mg protein/ml) were carried out in 100 mM KH_2PO_4 buffer at pH 7.4 and at 37° C for up to 30 min. Reactions were terminated by the addition of 2 vols cold acetonitrile. The mixture was centrifuged and the clear supernatant subjected to HPLC. Altex Ultrasphere-ODS reverse phase columns of 5 μ particle size, 4.6 mm x 25 cm, fitted with a precolumn (4.6 mm x 5 cm) were used. The mobile phase was acetonitrile:100 mM sodium acetate (85:15, v/v) containing 0.1% triethylamine (final pH 5.6). The flow rate was 2 ml/min and detection was at 254 nm. Monoamine oxidase activity was measured spectrophotometrically using benzylamine as substrate (13). Preparative scale incubation studies were run in a similar way except that the protein concentration of the mitochondrial preparations was 5 mg protein/ml and the concentration of MPTP was 1.0 mM. Isolation of the metabolic product was achieved by preparative HPLC [mobile phase acetonitrile:100 mM acetic acid (85:15, v/v) containing 0.17% triethylamine, pH 5.6] using the analytical column and 12 x 400 μl injections at a flow rate of 2 ml/min. The fractions eluting between 3.24 and 5.20 min were collected and the solvent removed by evaporation at 40° C under a stream of nitrogen. The residue was dissolved in acetonitrile (for CI mass spectral analysis) or acetonitrile-d_3 for ^{1}H NMR analysis. A small amount (ca 1 μg) of the isolate was applied to the ceramic tip of the direct insertion probe of an AEI MS 902S mass spectrometer modified for CI mass spectral analysis. Spectra were obtained at 8 kV using isobutane (0.5 torr) as reagent gas over a temperature range of 85 to 200° C. The ^{1}H NMR spectrum was obtained at 240 MHz on a custom built instrument. The amount of sample was estimated to be ca 100 μg; 200 transients were collected.

RESULTS AND DISCUSSION. Reverse-phase analytical HPLC was used to estimate the extent to which MPTP is metabolized by incubation mixtures of whole rat brain homogenates and subcellular fractions. Significant brain metabolic activity was found and proved to be concentrated in the crude mitochondrial fraction (specific activity 0.91 ± 0.02 nmoles/mg protein/min) which accounted for over 60% of the total activity in the homogenate. No metabolism of MPTP was observed if mitochondrial fractions were boiled prior to incubation.



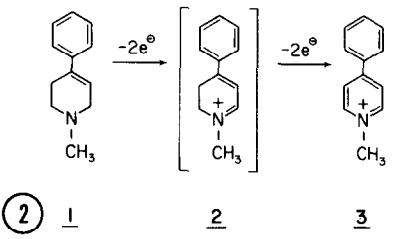


Figure 1. HPLC tracings of MPTP and its major metabolite. Rat brain mitochondrial fractions (1.5mg protein/ml) in pH 7.4 phosphate buffer were incubated with MPTP (0.1 M) at 37°C. Acetonitrile extracts were analyzed as described in METHODS. Dashed line--0 min; dot-dashed line--15 min; solid line--30 min.

Figure 2. Brain mitochondrial metabolism of MPTP (1) results in the formation of the 4-electron oxidation product, the 1-methyl-4-phenylpyridinium species 3. The dihydropyridinium species 2 is suggested as a possible intermediate in the oxidation of MPTP which appears to be mediated by type B MAO.

As shown in Figure 1, metabolism of MPTP leads to the formation of a UV absorbing peak which has a shorter retention time than that of MPTP on reverse-phase HPLC chromatography. The material present in this metabolism-derived peak was isolated by preparative HPLC and subjected to direct insertion probe CI mass spectral analysis. The principal ion observed in the spectrum was at mass 156 which is consistent with the protonated molecular ion of 4-phenylpyridine. ^{1}H NMR analysis of the product, however, clearly documented that the metabolite had retained the N-methyl substituent since a sharp singlet integrating for 3 protons was present in the spectrum at 4.2 ppm. Four sets of overlapping multiplets occuring in the aromatic region of the spectrum integrated *in toto* for 9 protons and could be readily assigned to the proton signals of the phenyl and pyridine moieties. Based on the ^{1}H NMR spectral data we have assigned the structure of this product as the 1-methyl-4-phenylpyridinium species (3, Figure 2). The absence of an ion corresponding to this species in the CI mass spectrum is consistent with the lack of volatility of this positively charged compound. The presence of the ion at mass 156 may be rationalized in terms of a thermolytic process leading to N-demethylation of the pyridinium compound to yield the volatile 4-phenylpyridine species which would undergo protonation under CI mass spectral conditions.

Table 1. Effects of MAO inhibitors on MPTP metabolism. The inhibitors (10^{-7} M) were pre-incubated with rat brain mitochondrial fractions (2mg protein/ml) in phosphate buffer (pH 7.4) for 5 min prior to the addition of MPTP (0.1mM). MPTP concentrations remaining at the end of the 30 min incubation period were estimated by HPLC as described in METHODS.

Inhibitor	MPTP metabolism (nmoles/mg protein/30 min)	% Inhibition
Control	18.5 ± 3.0	----
Pargyline	0	100
Deprenyl	2.9 ± 2.3	85
Clorgyline	19.5 ± 2.4	0

The chromatographic properties of synthetic 1-methyl-4-phenylpyridinium iodide was identical to that of the metabolite on both HPLC and silica gel TLC. The CI mass and ^{1}H NMR spectral characteristics of the synthetic compound also were identical to those of the product isolated from the mitochondrial incubation mixtures. This product recently has been identified by fast atom bombardment mass spectrometry in brain tissue samples isolated from monkeys which had been administered MPTP intravenously and which displayed a fully developed parkinsonian syndrome (14).

Brain mitochondrial metabolism of MPTP was decreased by co-incubation with inhibitors of MAO (Table 1). Pargyline, a selective inhibitor of type B MAO, completely blocked MPTP metabolism at a concentration of 10^{-7} M. Deprenyl, which also displays selective type B inhibition properties, inhibited MPTP metabolism by 85% under similar conditions. Clorgyline, a selective inhibitor of type A MAO, had no effect on the metabolism of MPTP. Thus it would appear that metabolism of MPTP by brain mitochondrial enzymes is catalyzed by type B MAO. Additional support for this suggestion comes from the observation that MPTP competitively inhibits the metabolism of benzylamine (a type B MAO substrate) by rat brain mitochondrial enzymes. With benzylamine as substrate at a concentration of 160 μM (its apparent K_M), MAO activity was inhibited approximately 50% by MPTP at 60 μM.

The formation of the 1-methyl-4-phenylpyridinium species from MPTP represents a 4-electron oxidation process. This process presumably takes place

through intermediate oxidation species including the dihydropyridinium compound (2, Figure 2), a 2-electron oxidation product. This postulated intermediate is equivalent to the reactive iminium species formed by the oxidative metabolism of other cyclic tertiary amines (5,6,8). The potential exists, therefore, for metabolic activation of MPTP by brain MAO to form electrophilic intermediates which may react with nucleophilic functionalities of neuronal macromolecules. Such bioactivation may be the initial chemical reaction in the chain of biochemical events leading to the neurotoxic actions of MPTP. Recent observations that decreases in dopamine and its principal metabolite 3,4-dihydroxyphenylacetic acid in neostriatum rodent brains caused by MPTP can be prevented by administration of pargyline add suport to this conjecture (15).

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