Studies on the mechanism of MPTP oxidation by human liver monoamine oxidase B

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The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its deuterated analogues were oxidized to their corresponding dihydropyridinium species ($MPDP^+$) by preparations of pure human liver MAO B:monoclonal antibody complex to investigate the mechanism of MPTP activation. Lineweaver-Burk plots of initial reaction rates revealed that the $K_{m,app}$ values for the various deuterated MPTP analogues were similar to those of MPTP. In contrast, $V_{max,app}$ values were substantially decreased by substitution of deuterium for hydrogen on the tetrahydropyridinium ring, especially at C-6. Deuterium substitution on the *N*-methyl group alone did not significantly reduce $V_{max,app}$. These studies support the interpretation that oxidation of MPTP at the C-6 position on the tetrahydropyridine ring is a major rate-determining step in its biotransformation by MAO B.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine *Neurotoxin* *Monoamine oxidase* *(Human liver)* *Deuteration* *Oxidation mechanism*

1. INTRODUCTION

Several lines of evidence have shown that activation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by monoamine oxidase B (EC 1.4.3.4) (MAO B) is a crucial step in generating neurotoxicity in mammals [1–5]. Oxidation of MPTP by MAO B has been demonstrated in a variety of species and tissues [1,4,6–8], and the enzyme-catalyzed formation of $MPDP^+$ has been established [6,9]. However, the specific rate-determining step in this process remains unknown. For example, one or more uncharged species of MPDP could be formed initially, followed by isomerization to $MPDP^+$. In an attempt to address this issue, we have synthesized 4 deuterated compounds of MPTP and compared their initial rates of oxidation by purified human liver MAO B with MPTP.

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2. MATERIALS AND METHODS

All MPTP deuterated analogues were synthesized according to [10]. Melting points were determined on a Fisher-Johns apparatus and are corrected. Electron impact mass spectra (EIMS) were obtained on a VG-Micromass 7070 F spectrometer with a DS 2050 data system. 4-Phenylpyridine, sodium borodeuteride-d_4 (98% D) and methyl alcohol-d (99.5% D) were purchased from Aldrich.

MPTP is readily obtained from 4-phenylpyridine (**1**) by quaternization with methyl iodide to methoiodide **2**, and reduction of the latter with

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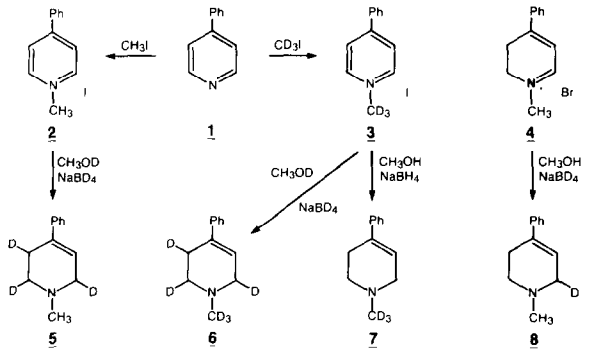
sodium borohydride in methanol [10]. Reduction of $MPDP^+$ (**4**) with sodium borohydride also yields MPTP [5]. The deuterated analogues of MPTP shown in scheme 1 were obtained using trideuteromethyl iodide as the quaternizing agent and sodium borodeuteride for reduction. Reduction of **2** with sodium borodeuteride gave 2,3,6-trideuterated compound **5** (HCl salt, m.p. 257°C; MS, *m/e* 176). Quaternization of **1** with trideuteromethyl iodide gave quaternary methoiodide **3** (m.p. 167–168°C), which was reduced with sodium borohydride to tetrahydropyridine **7** deuterated in the methyl group (HCl salt, m.p. 257°C; MS, *m/e* 176), and with sodium borodeuteride to hexadeutero compound **6** (HCl salt, m.p. 257–258°C; MS, *m/e* 179). Reduction of **4** with sodium borodeuteride in methanol afforded 6-deutero-MPTP **8** (HCl salt; m.p. 255–256°C, MS, *m/e* 174).

Human liver MAO B was prepared using a monoclonal antibody (MAO B-1C2) specific for the B form of the enzyme [11,12] according to Patel et al. [13]. The enzyme-antibody complex, which is catalytically active, was assayed at 30°C using various concentrations of test compounds in 0.5 ml of 25 mM sodium phosphate, pH 7.4. MPTP and its deuterated analogues were dissolved in buffer and their concentrations determined by measuring the absorbance at 243 nm of diluted stock solutions using a molar extinction coefficient of 1.30×10^4. Initial reaction rates were measured during the first 5 min by monitoring the increase in absorbance at 340 nm for the formation of the appropriate dihydropyridinium species using a Beckman DU7U spectrophotometer. $MPDP^+$ has a molar extinction coefficient of 1.30×10^4 in 25 mM sodium phosphate buffer, pH 7.4.

SCHEME I



3. RESULTS

The kinetic parameters obtained from Lineweaver-Burk plots for the oxidation of MPTP and its deuterated analogues to the dihydropyridinium species by pure human liver MAO B:MAO B-1C2 are shown in table 1. Values obtained for $K_{m,app}$ of MPTP and its deuterated analogues were similar, indicating that deuterium labeling did not alter the apparent affinity of the enzyme for these analogues. In contrast, compounds containing deuterium substitutions on the

Table 1

Kinetic parameters of MPTP and its deuterated analogues by pure human liver MAO B:MAO B-1C2 from two individuals

Compound	$K_{m,app}$ (μM)	$V_{max,app}$ (nmol·min^{-1}·mg^{-1})
MPTP	65.8, 75.9	99.6, 84.0
5	67.1, 82.0	60.1, 46.4
6	71.1, 96.9	58.3, 44.6
7	50.7, 69.7	90.8, 71.7
8	59.2, 85.3	67.1, 55.9

Human liver MAO B:MAO B-1C2, prepared from two individuals, was assayed spectrophotometrically by measuring the increase in absorbance at 340 nm with 6 different concentrations of MPTP or its deuterated analogues (50–333 μM). Values for $K_{m,app}$ and $V_{max,app}$ were obtained by linear regression analysis of $1/V$ vs $1/[S]$. Reactions were carried out in 0.5 ml of 25 mM sodium phosphate buffer, pH 7.4, with either 18.2 μg enzyme (preparation 1, left column) of 12.6 μg enzyme (preparation 2, right column) at 30°C and started by the addition of substrate. Initial rates were obtained with duplicate or triplicate determinations for each concentration from 0 to 5 min by monitoring the absorbance at 340 nm using a Beckman DU7U spectrophotometer. Correlation coefficients (>0.99) were obtained for all plots. It should be noted that liver MAO B from these individuals gave $K_{m,app}$ values of 65.8 and 75.9 μM, respectively, for MPTP. Previous studies [6] with a different preparation gave a $K_{m,app}$ of 316 μM, a result which indicates that significantly different enzyme affinities for substrate may occur among individuals

tetrahydropyridine ring displayed significant reductions in the $V_{max,app}$ compared to MPTP. Reduction (approx. 33%) of the $V_{max,app}$ of MPTP was observed when the deutero label was restricted to the 6 position of the tetrahydropyridine ring (**8**). Further deuterium substitutions on the tetrahydropyridine ring (**5**) and on the ring and methyl group (**6**) resulted in only slightly additional decreases in the $V_{max,app}$ compared to **8**. Little or no reduction of $V_{max,app}$ was observed with methyl deuterated compound (**7**).

4. DISCUSSION

MAO B catalyzes the oxidative deamination of neuroactive and vasoactive amines in the central nervous system and peripheral tissues. Studies on the mechanism of enzyme action indicate that primary amines are first oxidized to imines, which are then hydrolyzed to their corresponding aldehydes and ammonia [14]. However, MPTP is a tertiary cyclic amine, and the mechanism of its oxidation is different. Increase of the absorption to 340 nm indicates that the tertiary amine, MPTP, is oxidized to the iminium salt, $MPDP^+$.

The oxidation of the deuterated forms of MPTP shows differential effects on $V_{max,app}$ whereas no apparent effect on the $K_{m,app}$ was observed. The fact that the $K_{m,app}$ was not significantly altered indicates that the affinity of MAO B for the deuterated substrates was unaffected. The reduction in the $V_{max,app}$ for those compounds containing the deutero label on the tetrahydropyridine ring, i.e. **5**, **6**, and **8**, however, is indicative of a deuterium effect. Only **7**, with the deutero label on the methyl group, was oxidized to the same extent as MPTP. The reduction of the $V_{max,app}$ for **8** was about the same as that of **5** and **6**. Our results support the interpretation that oxidation of MPTP at the C-6 position of the tetrahydropyridine ring is a rate-limiting step in the formation of $MPDP^+$. Subsequent rearrangement of $MPDP^+$ to uncharged isomers, which are highly labile, could occur.

Previous studies have shown that $MPDP^+$ is not a substrate for MAO B, but undergoes chemical disproportionation to MPTP and MPP^+ [6,9]. This charged pyridinium species can compete for the uptake of dopamine in striatal synaptosomal preparations [15,16]. Furthermore, following in vivo administration of MPTP, MPP^+ accumulates in neurons in the substantia nigra and is thought to be correlated with cytotoxicity [3,17].

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