Metabolism of the neurotoxin in MPTP by human liver monoamine oxidase B

Richard R. Fritz, Creed W. Abell[†], Nutan T. Patel, Wieslaw Gessner[*+] and Arnold Brossi[*]

*Department of Human Biological Chemistry and Genetics, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550, and *Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205, USA*

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was oxidized to dihydropyridine $MPDP^+$ and pyridine MPP^+ by preparations of monoamine oxidase B (MAO B), including pure human liver MAO B monoclonal antibody complex $K_{m,app}$ values for MPTP and benzylamine, a preferred MAO B substrate, were 316 and 64 μM, respectively 4-Phenyl-1,2,3,6-tetrahydropyridine (PTP), the nor derivative of MPTP, was also a substrate ($K_{m,app} = 221$ μM) $MPDP^+$, MPTP, and MPP^+, but not PTP, were found to be irreversible inhibitors of MAO B Our studies support the hypothesis that MPTP is oxidized in primate brain by MAO B to $MPDP^+$, which is then converted to MPP^+, a major metabolite found in the substantia nigra

MPTP $MPDP^+$ MPP^+ Monoamine oxidase   Substantia nigra   Dopamine inhibition

1. INTRODUCTION

Several studies suggest that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) must be metabolized to produce Parkinson-like symptoms [1–4]. Monoamine oxidase (MAO, EC 1.4.3.4), the major intracellular enzyme which degrades biogenic amines in mammals [5], is thought to play an important role in this process. Chiba et al. [1] found that in rat brain mitochondrial fractions, MPTP can be converted by MAO B, one of the two forms of this enzyme, to its corresponding pyridinium derivative, MPP^+, a major metabolite in primates [6–7]. Furthermore, Langston et al. [2] have shown that pretreatment of primates with the MAO B inhibitor, deprenyl, prevents the development of MPTP-induced parkinsonism, while studies by Heikkila et al. [3] demonstrate in mice that clorgyline, an MAO A inhibitor, does not pre-

vent the dopamine-depleting effect of the drug.

Our studies [8] show that MPTP is metabolized to MPP^+ by MAO B via the dihydropyridine $MPDP^+$. Simultaneously, Salach et al [9] demonstrated that homogeneous preparations of bovine liver MAO B and human placental MAO A oxidize MPTP with K_m values similar to those of benzylamine. Both forms of the enzyme were irreversibly inactivated when incubated with high concentrations (1–5 mM) of MPTP. Collectively, these data led us to examine the kinetic properties of human MAO B with MPTP as substrate, since highly purified preparations of human or other primate MAO B had not previously been studied.

2. MATERIALS AND METHODS

One source of enzyme used in these studies was pure, catalytically active MAO B:monoclonal antibody complex (MAO B:MAO B-1C2) prepared from human autopsy liver as described by Patel et al. [10], using a well characterized monoclonal an-

[†] To whom correspondence should be addressed
[+] Visiting Scientist from A. Mickiewicz University, Poznan, Poland

tibody (MAO B-1C2) which recognizes human MAO B but not MAO A [11,12]. MAO B:MAO B-1C2 was studied because MAO B has not yet been separated from MAO B-1C2 in catalytically active form, nor have preparations of pure human MAO B with high catalytic activity been obtained by other methods. Liver was the most appropriate source of human MAO B because autopsy brain is difficult to obtain. The substrate preferences and molecular activities of human MAO B in liver and brain appear to be essentially identical [13]. Crude extracts of human liver mitochondria and partially purified bovine liver MAO B were prepared and studied as described in [8,9].

MPTP and 4-phenyl-1,2,3,6-tetrahydropyridine (PTP) were purchased from Aldrich. MPDP$^+$ and MPP$^+$ were prepared and characterized as in [8] (see fig.1 for structures). These compounds exhibit different UV spectra, and the oxidation of MPTP [8] and PTP (unpublished) into their corresponding dihydropyridinium derivatives can be followed spectrophotometrically at 340 nm (E^M = 1 30 × 10^4) in aqueous solutions.
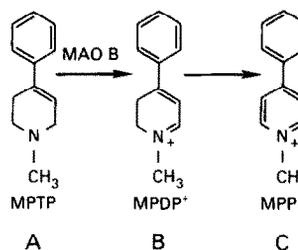

Fig.1 Structures of MPTP, MPDP$^+$ and MPP$^+$

Table 1

Kinetic parameters of MPTP, PTP and benzylamine oxidation by human and bovine liver MAO preparations

Source of enzyme	Substrate	$K_{m,app}$ (μM)	$V_{max,app}$ (nmol/min per mg)
Partially purified bovine liver MAO B	MPTP	179	34 6
	benzylamine	128	76 9
Human liver mitochondrial extracts (1 5% Triton X-100)	MPTP	152	2 47
	PTP	282	4 17
	benzylamine	34	11 2
Pure human liver MAO B·MAO B-1C2	MPTP	316	229
	PTP	221	196
	benzylamine	64	880

Partially purified bovine MAO B was assayed spectrophotometrically in triplicate with 9 different concentrations of MPTP (40–650 μM, A_{340nm}) and benzylamine (25–250 μM, A_{250nm}) $K_{m,app}$ and $V_{max,app}$ values were calculated by linear regression analysis of the data plotted as $1/v$ against $1/S$ The correlation coefficients of the regression lines were 0 99 for MPTP and 0 98 for benzylamine and the values of the triplicate points were ± 8% SD Protein concentrations used for the enzyme activity measurements were 11 μg protein per sample for both substrates Human liver mitochondrial extracts were assayed in triplicate for activity at 9 concentrations of MPTP (20–200 μM), PTP (20–480 μM) and benzylamine (20–480 μM) Values for $K_{m,app}$ and $V_{max,app}$ were calculated as stated above with correlation coefficients of 0 99, 0.97, 0.98, respectively. The means for the triplicates were ± 5% SD for benzylamine and MPTP and ± 10% SD for PTP. Protein concentrations used for these measurements were 242 μg for MPTP and PTP and 48 μg for benzylamine per sample analyzed Pure human liver MAO B MAO B-1C2 was assayed at 9 concentrations in triplicate of MPTP (40–400 μM), PTP (40–400 μM) and benzylamine (10–160 μM). $K_{m,app}$ and $V_{max,app}$ values were obtained as described above, correlation coefficients were 0 98, 0 98, 0 99, respectively. The means of the triplicates were ± 5% SD for MPTP and PTP and ± 8% SD for benzylamine MAO B protein concentrations were 6.7 μg per sample for MPTP and PTP and 0 67 μg per sample for benzylamine

3. RESULTS

Table 1 shows the kinetic properties for the oxidation of MPTP and PTP to their corresponding dihydropyridinium analogs by pure human liver MAO B: MAO B-1C2 and detergent extracts of human liver mitochondria. $K_{m,app}$ values for MPTP were similar, although not identical, in these 2 preparations. $K_{m,app}$ values for benzylamine, however, were approx 5-fold lower than for MPTP A comparison of MPTP and benzylamine oxidation by partially purified bovine MAO B (about 80% pure as judged by examination on SDS gels), which was prepared according to Salach [14], gave similar $K_{m,app}$ values (179 and 129 μM, respectively). In all MAO B preparations studied, however, benzylamine was oxidized at a 2–4-fold faster rate than MPTP.

MAO B catalytic activity declined progressively when MPTP was used as substrate, suggesting that the enzyme was being inactivated. Therefore, the effects of MPTP, MPDP$^+$, MPP$^+$ and PTP on benzylamine oxidation of MAO B: MAO B-1C2 were assessed as a function of time. Fig.2 shows that after a short initial lag, enzyme inactivation by MPTP followed first-order kinetics. MPDP$^+$ and MPP$^+$ also inactivated the enzyme complex, but PTP (1 mM) had no effect. The pseudo first-order rate constants for the inactivation of MAO B: MAO B-1C2 were 3.92×10^{-2} min^{-1} for MPTP (1 mM), 3.48×10^{-2} min^{-1} for MPDP$^+$ (0.2 mM), and 1.77×10^{-2} min^{-1} for MPP$^+$ (10 mM) Attempts to recover activity by overnight dialysis against buffer were unsuccessful. Furthermore, incubation of [^{3}H]MPTP with MAO B: MAO B-1C2 resulted in the incorporation of tritium into protein, which could not be removed by extensive washing with buffer.

These results support the concept that MPTP can be oxidized by human MAO B to MPDP$^+$, an intermediate in the formation of the metabolite MPP$^+$. Using crude preparations of MAO B, we have shown that MPTP is oxidized to MPDP$^+$ at a 5-fold faster rate than MPDP$^+$ is converted to MPP$^+$ [8]. However, since MPDP$^+$ chemically undergoes rapid disproportionation at the pH of the incubation mixture [15,16] and has spectral properties in water (max 343 nm) which are similar to those of MPP$^+$ (max. 293 nm), it is presently not possible to determine whether the
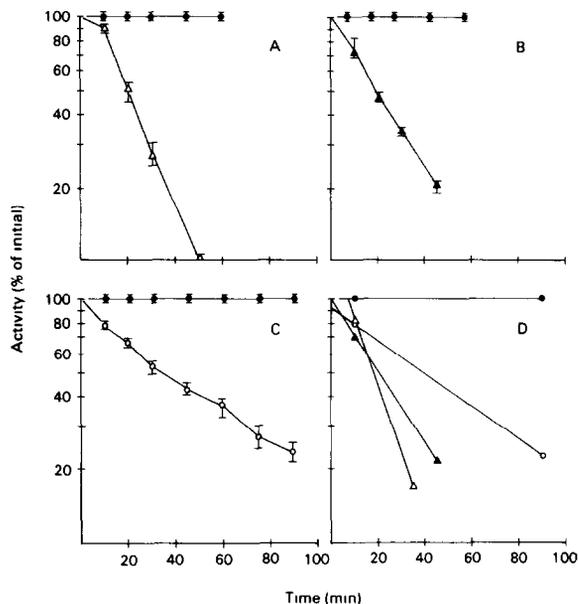

Fig 2. Irreversible inactivation of pure human liver MAO B. MAO B-1C2 by MPTP (1 mM, panel A), MPDP$^+$ (0 2 mM, panel B), and MPP$^+$ (10 mM, panel C) MAO B.MAO B-1C2 (62 μg protein) was suspended in 50 mM KH$_2$PO$_4$/K$_2$HPO$_4$ buffer, pH 7.4, and incubated at 30°C with 1 mM MPTP, 0.2 mM MPDP$^+$, and 10 mM MPP$^+$, respectively At the times indicated, triplicate 10-μl aliquots were removed, added to the assay cuvettes, and diluted to 1 0 ml with the assay solution, which contained 3 mM benzylamine in 50 mM potassium phosphate buffer, pH 7 4 Initial rates were determined from 0 to 2 min by measuring the increase in absorption at 250 nm using a Beckman DU-7U spectrophotometer Panel D shows linear regression analyses of the data; the correlation coefficients in all cases were >0 99 (●) Control or 1 mM PTP (panel A), (△) 1 mM MPTP, (▲) 0 2 mM MPDP$^+$; (○) 10 mM MPP$^+$ The calculated rate constants k (min^{-1}) were 3 92 × 10^{-2} for 1 mM MPTP, 3 48 × 10^{-2} for 0.2 mM MPDP$^+$ and 1 77 × 10^{-2} for 10 mM MPP$^+$ Overnight dialysis vs phosphate buffer after 100-fold dilution did not affect the activity measurements

formation of MPP$^+$ from MPDP$^+$ is the result of this chemical transformation or additional enzymatic oxidation.

MPTP and its oxidation products were found to inhibit MAO B irreversibly with potencies ranked as follows: MPDP$^+$ > MPTP > MPP$^+$. In contrast, PTP did not inactivate MAO B. The N-methyl substituent is clearly important for irreversible enzyme inhibition.

4. DISCUSSION

The relevance of MAO inactivation to the neurotoxic effects of MPTP in vivo is unclear. Patients treated for endogenous depression with irreversible inhibitors of MAO A and B (e.g., tranylcypromine) do not develop parkinsonian symptoms [17]. Furthermore, if irreversible inhibition of MAO B were the crucial event for neurotoxicity, many MAO B-containing neurons would presumably undergo similar damage. In fact, however, we have shown that MAO B is not found in the highly sensitive cells of the substantia nigra. Our localization studies of MAO A and MAO B in *Macaca cynomolgus* monkey brain, performed with MAO type-specific monoclonal antibodies, revealed that MAO B is localized largely in serotonergic neurons and not dopaminergic neurons, while MAO A is localized in catecholaminergic regions, including the substantia nigra [18].

Given these patterns of distribution, we hypothesize that MAO B catalyzes the oxidation of MPTP to $MPDP^+$ and MPP^+ in serotonergic neurons which impinge either on dopaminergic cells in the substantia nigra or their terminals in the striatum, or in MAO B-containing, astrocytic glial cells. These metabolites may be transported out of these cells and taken up by neurons in the substantia nigra. After this work was completed, Javitch et al. [19] also proposed that MPP^+ may be produced in astrocytes and transported into neurons in the substantia nigra. Previous studies show that 48–72 h after MPTP administration to monkeys, MPP^+ accumulates in the substantia nigra, but decreases in surrounding regions of brain [20]. In rats, MPP^+ is transported into neurons through the dopamine uptake system in striatal preparations [21].

The biological target(s) of MPP^+ in neurons of the substantia nigra is unknown, but one possible candidate is MAO A. In vitro preparations of pure human placental MAO A converts MPTP to MPP^+, an irreversible inhibitor of this enzyme [9]; however, MPTP is a reversible competitive inhibitor of rat brain MAO A [22]. If MPP^+ is an effective in vivo inhibitor of primate neuronal MAO A, its accumulation in the substantia nigra could interfere with the function of this important amine-degrading enzyme and thereby contribute to specific neurotoxicity.

Collectively, the results of our studies of human MAO B and those of others using MAO B from other mammals [1–4,9] support the hypothesis that MPTP is oxidized by MAO B to compounds that can cause significant inhibition of both MAO A and MAO B. These molecular events could contribute to specific neuronal toxicity in the substantia nigra and lead to Parkinson's disease.

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