Further insight into the mode of action of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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Chemical reactions of $MPDP^+$, a recognized intermediate in the metabolic conversion of the neurotoxin MPTP by monoamine oxidase B into its major metabolite MPP^+, were studied.Addition of cyanide to $MPDP^+$ bromide in aqueous solutions afforded cyano-compound **5** which isomerized in the presence of silica gel into compound **6**. Both **5** and **6** when heated yielded a third isomer **7**. $MPDP^+$ bromide disproportionated into MPTP and MPP^+ in aqueous solution near neutral or slightly alkaline pH, a reaction which also occurred when $MPDP^+$ bromide was treated with an amine in dichloromethane solution. Disproportionation of $MPDP^+$ at physiological pH may be of biochemical significance, since formation of MPP^+ from $MPDP^+$ can occur non-enzymatically. MPTP, $MPDP^+$, and MPP^+ inhibited dopamine uptake in rat synaptosomal preparations with I_{50} values of 30, 37, and 3.4 μM, respectively. The competition of these compounds with dopamine for uptake sites in the membrane may contribute in part to the reduced levels of dopamine observed in animals treated with MPTP.

MAO oxidation *Neurotoxin* *MPTP* *Metabolism* *Dihydropyridine* *Dopamine uptake*

1. INTRODUCTION

The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) [1–4] is converted in vivo by brain tissue [5], and in vitro by monkey brain homogenates [6] and rat brain mitochondrial incubation mixtures [7,8] into the 1-methyl-4-phenylpyridinium species (MPP^+, **3**), a major metabolite of MPTP in primates which accumulates in brain tissue [5]. It was found [7,9–11] that pargyline and other MAO-B inhibitors block this conversion of MPTP, suggesting that oxidation of MPTP is catalysed by this enzyme. The hypothesis that 1-methyl-4-phenyl-2,3-dihydropyridinium species ($MPDP^+$, **2**) is an intermediate in the metabolic conversion of MPTP into MPP^+ [4,7] was recently proved in experiments with purified MAO-B from human [12] and bovine liver [12,13]. Formation of $MPDP^+$ was also demonstrated indirectly by cyanide addition, to trap reactive intermediates formed by incubation of MPTP with mouse liver microsomal preparations. Gas chromatography-mass spectrometry (GC-MS) showed in this case the presence of 3 different cyano-compounds [14].

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2. MATERIALS AND METHODS

Rat striatal synaptosomes were prepared by the method of Gray and Whittaker [15] and suspended in a medium containing 50 mM Tris-HCl (pH 7.4), 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose (physiological Tris). An incubation mixture containing test compound, [^{14}C]dopamine (0.89 μM, spec. act. 0.5 μCi/μmol), 0.02% ascorbic acid, and 0.125 mM nialamide in physiological Tris was prewarmed at 37°C

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for 5 min. [^{14}C]Dopamine uptake was initiated by addition of the freshly prepared synaptosomes (~0.2 mg P_2-protein) and the reaction mixture (total volume 1 ml) incubated at 37°C for 5 min. The reaction was stopped by chilling the incubation tubes in ice-water and the synaptosomes recovered by membrane filtration (Millipore HAWP, 0.45 μm). After washing the filters twice with ice-cold physiological Tris (5 ml each time), the radioactivity retained on the filters was determined by a Beckman LS 6800 Scintillation System in 7.5 ml Hydrocount (Baker). A parallel [^{14}C]dopamine uptake study at 0°C (ice bath) was carried out for each compound and the values subtracted from those obtained at 37°C. The net value represents the amount of specific [^{14}C]dopamine uptake. The percent inhibition of [^{14}C]dopamine uptake by the test compound was calculated and plotted over at least 6 different concentrations. The concentration that inhibits 50% of the synaptosomal [^{14}C]dopamine uptake is expressed as the I_{50} value.

MPTP was purchased as the free base from Aldrich, Milwaukee, WS. MPDP$^+$ (**2**) as bromide salt was obtained as described [12]. Addition of cyanide to MPDP$^+$ was performed as described by Gierson et al. [16]. Melting points were determined on a Fisher-Johns apparatus and are corrected. ^{1}H-NMR spectra were recorded using a Varian HR-220 or Varian XL-300 spectrometer with TMS as internal standard. Electron impact mass spectra (EIMS) were obtained on a VG-Micromass 7070 F spectrometer with a DS 2050 data system. UV spectra were measured with a Hewlett-Packard 8450A UV-VIS spectrometer. TLC plates were purchased from Analtech, Newark, DE and silica gel 60 (15–40 μm) was from Merck, Darmstadt, FRG.

3. RESULTS

Addition of cyanide to MPDP$^+$ bromide (**2**) in a two-phase system of dichloromethane-water [16] gave cyano-compound **5** [17], which isomerized in dichloromethane solution when passed through a silica gel column into the new isomer **6**. Cyano-compounds **5** and **6** were indistinguishable on TLC. The new isomer **6** had the following characteristics: m.p. 91–92°C (from isopropyl ether); EIMS, *m/z* 198 (M^+); ^{1}H-NMR δ 7.32, 6.11, 3.99, 3.45, 3.10, 2.73 and 2.50; UV (MeOH) λ_{max} 245 nm (ϵ = 14600). While cyano-compound **5** afforded **2** with perchloric acid [8], cyano-compound **6** is stable to acid at room temperature, but is converted, like **5**, upon heating to 150°C to isomer **7**, with the C=C double bond conjugated to the carbon atom bearing the cyano-substituent. Isomer **7**, obtained as an oil, showed the following characteristics: EIMS, *m/z* 198 (M^+); ^{1}H-NMR ($CdCl_3$) δ 7.26, 5.43, 3.57, 2.98, 2.86, 2.14 and 1.88; UV (MeOH) λ_{max} 275 nm (ϵ = 6700).

A solution of MPDP$^+$ bromide (**2**) in water (pH 5.0), when adjusted in argon atmosphere to pH 7 with sodium bicarbonate solution quantitatively gave **1** and **3** by disproportionation. This reaction could easily be followed by UV spectroscopy and a shift of the UV maximum from 343 nm to 293 nm characteristic for **3** in water [12].

The reaction mixture obtained by disproportionating 250 mg MPDP$^+$ bromide (**2**) in water afforded 76 mg MPTP (**1**) and 118 mg MPP$^+$ iodide (**3**), isolated from the aqueous phase after removal of MPTP with ether, adjusting the pH to 10 with sodium hydroxide, addition of sodium iodide and extraction with a mixture of dichloromethane-isopropanol (4:1).

Disproportionation also occurred when **2** was treated with diethylamine, triethylamine, or β-(3,4-dimethoxyphenyl)ethylamine in dichloromethane solution at 0°C. MPDP$^+$ bromide (**2**) did not react with dopamine in *aqueous* solutions over a wide pH range (5.0–7.6) since no formation of new catecholic products could be detected on TLC.

The effects of MPTP and its metabolites on

Table 1

Inhibition of synaptosomal dopamine uptake by MPTP, MPDP$^+$ and MPP$^+$

Compound	I_{50} (μM)
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	30
1-Methyl-4-phenyl-2,3-dihydropyridinium bromide (MPDP$^+$)	37
1-Methyl-4-phenylpyridinium bromide (MPP$^+$)	3.4

At least 6 different concentrations of each compound were tested for inhibition of [^{14}C]dopamine uptake into synaptosomes as described in the text

dopamine uptake in synaptosomal preparations are shown in table 1. MPTP and $MPDP^+$ blocked dopamine uptake in the 10^{-5} M range. MPP^+ was the most effective compound with an I_{50} value of 3.4 μM. In comparison, nomifensine, a potent inhibitor of dopamine re-uptake into presynaptic neuronal terminals [18], gave an I_{50} value of 0.1 μM.

4. DISCUSSION

Results of studies with the neurotoxin MPTP (**1**), which depletes the substantia nigra of dopamine, and causes toxic symptoms similar to those observed in Parkinson's disease in man, suggest that consequences of its metabolism are complex. The findings reported here, and corroborated by data obtained elsewhere, suggest that MPP^+ (**3**), a major metabolite found in the nigrostriatum of man and animals after exposure to MPTP, originates from the precursor $MPDP^+$ (**2**) or its dienamine tautomer. $MPDP^+$ is formed in vivo in an oxidation reaction catalyzed by MAO-B [12,13]. A study of the chemical reactivity of $MPDP^+$ and MPP^+ under physiological conditions seems, therefore, of paramount importance.

Data presented here show that the addition of cyanide, intended to trap the chemically reactive $MPDP^+$ [14] affords cyano-compounds **5** and **6** depending on reaction conditions. Cyano-compound **5** is converted into isomer **6** when exposed in dichloromethane solution to silica gel, and both **5** and **6** are converted into the thermodynamically most stable isomer **7** when heated to 150°C. Formation of **6** from **5** was not investigated in detail, and is assumed to have originated by abstraction of hydrogen cyanide from **5** and addition to the discretely formed iminium species **4** or its dienamine tautomer. The finding that $MPDP^+$ did not react with dopamine under physiological conditions makes it unlikely that $MPDP^+$ traps biogenic amines to cause an imbalance in amine levels, as has been suggested [4,7].

The observed disproportionation of $MPDP^+$ into MPTP and MPP^+ at physiological pH, also observed by Castagnoli et al. [8], and not affected in brain mitochondrial incubation mixtures when pargyline was added [8], supports the idea that physiological factors may favor disproportionation of $MPDP^+$. It should be noted that MPTP (**1**), a tertiary amine, is able to cross membranes and thus infiltrate neighboring neurons, undergoing there the same reaction cycles. MPP^+, on the other hand, is a quaternary species, which probably remains in neurons of origin until it is further metabolized; by doing so, however, it may block their functioning as suggested by Markey et al. [5]. MPP^+ is acutely more toxic than MPTP to mice after i.p. administration [19], and identification of its biological targets may be directly related to the development of Parkinson-like symptoms in animal and man. In this regard, it is of interest to note that Javitch and Snyder [20] recently found that MPP^+, but not MPTP, accumulates through the dopamine neuronal uptake system.

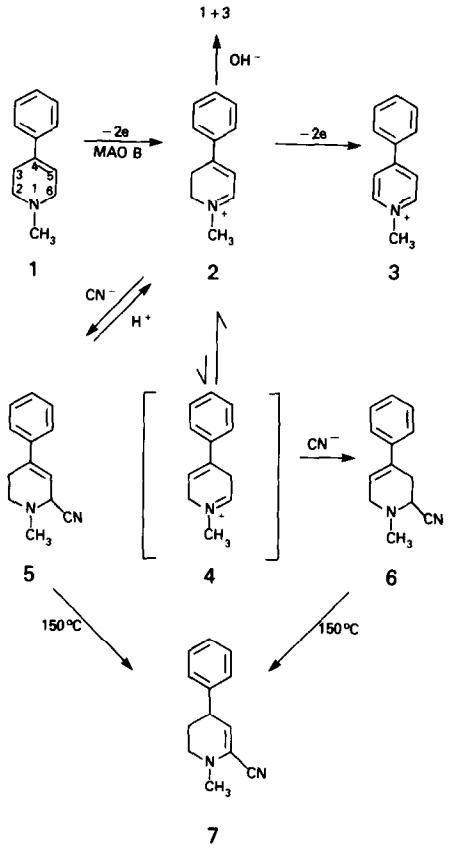

Fig.1.

Our finding that MPP^+, and to a lesser extent $MPDP^+$ and MPTP, inhibit dopamine uptake in synaptosomal preparations is consistent with

previous studies which show that MPTP inhibits dopamine uptake in synaptosomes from several species [21], and cultured rat pheochromocytoma cells [22]. Our finding that MPP^+, the product which accumulates in the substantia nigra, is the most potent derivative tested, may explain in part the reduced levels of dopamine in the nigrostriatum following MPTP administration.

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