# Serotonergic conversion of MPTP and dopaminergic accumulation of $MPP^+$

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[$^3$H]$MPP^+$ had lower $K_m$ and higher $V_{max}$ values for its accumulation in rat brain synaptosomes than did [$^3$H]MPTP. The kinetic parameters favored the uptake of [$^3$H]$MPP^+$ in the striatum to that in hypothalamus, whereas they were equally favorable for the uptake of [$^3$H]MPTP in both regions. Hypothalamic uptake of [$^3$H]MPTP and [$^3$H]$MPP^+$ was inhibited by desipramine, imipramine, norepinephrine, and serotonin. Striatal uptake of [$^3$H]$MPP^+$ and [$^3$H]MPTP was blocked by nomifensine and dopamine. These results support the concept that MPTP accumulates in serotonergic neurons where it is oxidized by monoamine oxidase B to $MPP^+$, which is released and then is selectively accumulated in dopaminergic neurons via the dopamine uptake system.

*MPTP* *$MPP^+$* *Neurotoxin* *Parkinsonism* *Dopamine uptake*

## 1. INTRODUCTION

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a nigrostriatal neurotoxin, produces parkinsonism in humans [1,2] and nonhuman primates [3,4]. The neurotoxicity of this substance is thought to be derived from a 1-methyl-4-phenylpyridinium species ($MPP^+$) [5,6], an oxidation product of MPTP which is formed from MPTP through a dihydropyridinium intermediate ($MPDP^+$) by monoamine oxidase (MAO, EC 1.4.3.4) [7–11]. Pretreatment of animals with the MAO B inhibitors, deprenyl and pargyline, effectively blocks this activation process [5,7,8], as well as MPTP-induced dopaminergic neurotoxicity [12–14] and the development of parkinsonian motor symptoms in primates [8,15]. In contrast, the MAO A inhibitor clorgyline has no protective effect [7,13]. Hence MAO B is thought to play a pivotal role in producing the specific neurotoxicity of MPTP.

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Javitch et al. [16] reported that mazindol, a dopamine uptake inhibitor, was as effective as pargyline in preventing MPTP-induced destruction of mouse dopaminergic neurons. Furthermore, Sundstrom and Jonsson [14] found that amfolenic acid, another dopamine uptake blocker, counteracted the MPTP-induced reduction of mouse striatal dopamine levels. These results suggest that the neurodegenerative effect of MPTP depends not only on its conversion to $MPP^+$ by MAO B, but also on the selective uptake of $MPP^+$ into nigrostriatal neurons.

A major unsolved question is the neuronal location of the conversion of MPTP to $MPP^+$. Autoradiography of [$^3$H]MPTP binding sites in rat brain demonstrated the most intense concentrations in the arcuate nucleus of the hypothalamus (900 fmol/mg), but lower concentrations in the substantia nigra (10–50 fmol/mg) [17]. These high-affinity, saturable and specific binding sites correspond to binding sites in rat brain of the MAO inhibitor, [$^3$H]pargyline [18]. Javitch and Snyder [19] also reported that [$^3$H]$MPP^+$, not [$^3$H]MPTP, accumulated into rat striatal synap-

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tosomes via a dopamine neuronal re-uptake system. Selective accumulation of $MPP^+$ in the substantia nigra of squirrel monkeys was also observed by Irwin and Langston [20]. Collectively, these results suggest that MPTP may be converted to $MPP^+$ in neurons outside the striatum or substantia nigra. The studies of Sundstrom and Jonsson [14] and Uhl et al. [21] implicate non-catecholaminergic neurons, while Javitch et al. [16] propose that $MPP^+$ originates from astrocytes. Direct evidence for the formation of $MPP^+$ in specified cell types and export to dopaminergic neurons has not yet been reported.

This paper presents evidence that [$^3$H]MPTP is taken up into serotonergic neurons, where it may be converted to $MPP^+$ by MAO B, while in contrast, $MPP^+$ is taken up into dopaminergic neurons by the dopamine re-uptake system. A hypothesis is also presented to explain the selective nigrostriatal neurotoxicity of MPTP.

## 2. MATERIALS AND METHODS

1-[*methyl*-$^3$H]MPTP and 1-[*methyl*-$^3$H]$MPP^+$ (acetate) (85 Ci/mmol) were obtained from New England Nuclear; and 5-hydroxy-[*side chain*-2-$^{14}$C]tryptamine creatinine sulphate (56 mCi/mmol), 1-[7,8-$^3$H]norepinephrine (32 Ci/mmol) and [8-$^{14}$C]dopamine (56 mCi/mmol) from Amersham; while MPTP, $MPDP^+$, and $MPP^+$ were synthesized in our laboratories [22]. Nomifensine maleate, desipramine hydrochloride and imipramine were generously supplied by Hoechst-Roussel Pharmaceuticals, Hoffman La Roche, and Ciba-Geigy, respectively. Other chemicals of reagent grade were obtained from commercial sources.

Rat (Sprague-Dawley) striatal and hypothalamic synaptosomes were prepared by a modification of the method of Gray and Whittaker [23,24]. Synaptosomes were 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 radiolabeled compound (50 nM [$^{14}$C]dopamine, 125 nM [$^{14}$C]serotonin, 0.25 nM [$^3$H]norepinephrine, 0.5 nM [$^3$H]MPTP, 0.5 nM [$^3$H]$MPP^+$, 0.02% ascorbic acid and 0.125 mM nialamide in physiological Tris was prewarmed with or without the test compound at 37°C for 5 min. The uptake of radiolabeled compound was initiated by addition of freshly prepared synaptosomes (~0.3 mg $P_2$-protein) and the reaction mixture (total volume 1 ml) was incubated at 37°C for an additional 5 min (striatal uptake) or 10 min (hypothalamic uptake for all radiolabeled compounds except [$^3$H]norepinephrine, which required 30 min incubation). A parallel study of the radiolabeled compound with and without the test agent was carried out at 0°C (ice bath) and the values subtracted from those obtained at 37°C to obtain active uptake values [25]. In routine uptake studies, values of non-$P_2$ blanks (assays without $P_2$-fractions at 37°C) were treated as controls and subtracted from the test values, because they were indistinguishable from blank values obtained by incubation at 0°C. The kinetic parameters ($K_m$ and $V_{max}$) of uptake were calculated from substrate saturation data and $IC_{50}$ values from drug inhibition isotherms. Protein content of the $P_2$-fractions was determined by the method of Lowry et al. [26].

## 3. RESULTS

The half-maximal uptake ($K_m$ values) of [$^3$H]$MPP^+$ into striatal and hypothalamic synaptosomes were 27 and 71%, respectively, of those for [$^3$H]MPTP uptake (table 1). The $K_m$ value for the uptake of [$^3$H]$MPP^+$ in striatum was one-half of that in hypothalamus. These results indicate that rat brain synaptosomes have a greater affinity for $MPP^+$ than for MPTP, and that $MPP^+$ is more likely to accumulate in striatum than in hypothalamus. In striatum, the $K_m$ value for [$^3$H]$MPP^+$ was approx. 40% of that for [$^{14}$C]dopamine. In hypothalamus, the $K_m$ value for [$^3$H]MPTP was approx. 88% of the $K_m$ for [$^3$H]norepinephrine, but was 4-fold of that for [$^{14}$C]serotonin. These data suggest that $MPP^+$ may compete with dopamine uptake in striatum, while MPTP may compete with norepinephrine uptake in hypothalamus.

The maximal rates of uptake ($V_{max}$ values) of [$^3$H]$MPP^+$ in both brain regions were approx. 3–5-fold higher than those observed for [$^3$H]MPTP (table 1). The $V_{max}$ for uptake of [$^3$H]$MPP^+$ in striatum was approx. 2-fold of that in hypothalamus, whereas the $V_{max}$ for uptake of [$^3$H]MPTP in striatum was only approx. 40% higher than that in hypothalamus. Compared to

Table 1

Kinetic parameters of the uptake of radiolabeled biogenic amines, MPTP, and $MPP^+$ in rat brain synaptosomes

| Radiolabeled compound | Brain region | $K_m$ ($\mu$M) | $V_{max}$ (pmol/min per mg) |
|---|---|---|---|
| [$^3$H]MPTP | striatum | 0.73 | 1.38 |
| | hypothalamus | 0.58 | 0.99 |
| [$^3$H]$MPP^+$ | striatum | 0.20 | 7.35 |
| | hypothalamus | 0.41 | 3.28 |
| [$^{14}$C]Dopamine | striatum | 0.49 | 42.78 |
| | hypothalamus | 0.24 | 6.58 |
| [$^3$H]Norepinephrine | striatum | 0.21 | 5.27 |
| | hypothalamus | 0.66 | 7.59 |
| [$^{14}$C]Serotonin | striatum | 0.19 | 9.57 |
| | hypothalamus | 0.13 | 4.35 |

All radiolabeled compounds (0.05–0.8 $\mu$M) were incubated at 37°C for 5 min (striatal uptake) or 10 min (hypothalamic uptake) with approx. 0.3 mg of freshly prepared $P_2$-fraction in physiological Tris containing 0.02% ascorbic acid and 0.125 mM nialamide. Control samples were incubated in the absence of the $P_2$-fraction. See section 2 for experimental procedures. $K_m$ and $V_{max}$ values were obtained by plotting the reciprocal of the uptake velocity vs the reciprocal of the concentration of the radiolabeled compound

the $V_{max}$ values for uptake of biogenic amines in striatum, the rate of [$^3$H]$MPP^+$ uptake was 17, 139 and 77% of those for [$^{14}$C]dopamine, [$^3$H]norepinephrine and [$^{14}$C]serotonin, respectively. Similarly, the $V_{max}$ value for uptake of [$^3$H]MPTP in hypothalamus was 15, 13 and 23% of those for [$^{14}$C]dopamine, [$^3$H]norepinephrine and [$^{14}$C]serotonin, respectively. These results indicate that $V_{max}$ values for uptake of [$^3$H]$MPP^+$ and [$^3$H]MPTP in rat brain synaptosomes are generally lower than the corresponding rates of uptake of biogenic amines.

Tables 2 and 3 present results from an extensive series of studies on the inhibition by drugs of the uptake of radiolabeled MPTP, $MPP^+$ and biogenic amines in rat striatal and hypothalamic synaptosomes. Nomifensine, a dopamine uptake blocker, and dopamine more effectively inhibited the uptake [$^3$H]$MPP^+$ than the uptake of [$^3$H]MPTP in striatal synaptosomes (table 2).

Table 2

Inhibition of the uptake of [$^{14}$C]dopamine, [$^3$H]MPTP and [$^3$H]$MPP^+$ into rat striatal synaptosomes

| Compound | $IC_{50}$, $\mu$M | | |
|---|---|---|---|
| | [$^{14}$C]Dopamine | [$^3$H]MPTP | [$^3$H]$MPP^+$ |
| Dopamine | 0.51 | 1.80 | 0.03 |
| Nomifensine | 0.08 | 0.45 | 0.04 |
| $MPP^+$ | 0.43 | 0.13 | 0.02 |
| MPTP | 5 | 2 | 6 |
| $MPDP^+$ | 13 | 2.5 | 0.22 |

Radiolabeled compounds (50 nM [$^{14}$C]dopamine, 0.5 nM [$^3$H]MPTP and 0.5 nM [$^3$H]$MPP^+$ were incubated with at least 6 concentrations of the test agent in the presence of freshly prepared striatal synaptosomes (~0.3 mg). The amounts of the active uptake of the radiolabeled compound into synaptosomes were calculated and plotted against the concentrations of the test agent to obtain the $IC_{50}$ values. Values presented are the mean of duplicate assays

Table 3

Inhibition of the uptake of [$^{14}$C]serotonin, [$^{3}$H]norepinephrine, [$^{3}$H]MPTP and [$^{3}$H]MPP$^{+}$ into rat hypothalamic synaptosomes

| Compound | $IC_{50}$, $\mu$M | | | |
|---|---|---|---|---|
| | [$^{14}$C]Serotonin | [$^{3}$H]Norepinephrine | [$^{3}$H]MPTP | [$^{3}$H]MPP$^{+}$ |
| Serotonin | ≤0.1 | 7.2 | 3.7 | 3.5 |
| Norepinephrine | ≤22.5 | 0.16 | 1.4 | ≤0.5 |
| Desipramine | ≥3.5 | ≤0.0035 | 0.088 | 0.0015 |
| Imipramine | 0.25 | ≤0.032 | 0.13 | 0.011 |
| MPTP | 1.5 | 1.2 | 0.80 | 0.23 |
| MPDP$^{+}$ | 87 | 0.80 | 0.13 | 0.58 |
| MPP$^{+}$ | 12.5 | 0.22 | 0.35 | 0.14 |

Radiolabeled compounds (125 nM [$^{14}$C]serotonin, 0.25 nM [$^{3}$H]norepinephrine, 0.5 nM [$^{3}$H]MPTP and 0.5 nM [$^{3}$H]MPP$^{+}$) were incubated with the test agent and hypothalamic synaptosomes (~0.3 mg). $IC_{50}$ values were obtained from the drug inhibition isotherms (see table 2). Values presented are the mean of duplicate assays

These 2 drugs also inhibited the striatal uptake of [$^{14}$C]dopamine, but with less potency. Table 3 shows that desipramine, a norepinephrine uptake blocker, was the most effective blocker of the uptake of both [$^{3}$H]MPTP and [$^{3}$H]MPP$^{+}$ in hypothalamus. Imipramine, a serotonin and norepinephrine uptake blocker, also inhibited the accumulation of tritiated MPTP and MPP$^{+}$ in hypothalamus, but with no more than 68% of the potency of desipramine. Norepinephrine and serotonin inhibited hypothalamic uptake of [$^{3}$H]MPTP and [$^{3}$H]MPP$^{+}$ with $IC_{50}$ values at the $\mu$M range (≤6% of the potency of desipramine). Table 3 also shows that the $IC_{50}$ values obtained by those 7 compounds are generally lower for [$^{3}$H]MPP$^{+}$ uptake than for [$^{3}$H]norepinephrine and [$^{14}$C]serotonin uptake in the hypothalamus.

## 4. DISCUSSION

Although MPTP must be converted to MPP$^{+}$ by MAO B to become cytotoxic [5–15], the basis of the selective nigrostriatal neurotoxicity of this compound remains unknown. The present finding of serotonergic conversion of MPTP to MPP$^{+}$ and dopaminergic accumulation of MPP$^{+}$ provides a model to explain the selective neurodegenerative effect of MPTP. Fig.1 summarizes our hypothesis, which is based on data from the rat brain synaptosomal system.

We propose that MPTP accumulates in the striatum and hypothalamus at equal rates. The accumulation of MPTP in the hypothalamus probably occurs via norepinephrine and serotonin re-uptake systems, because desipramine, im-

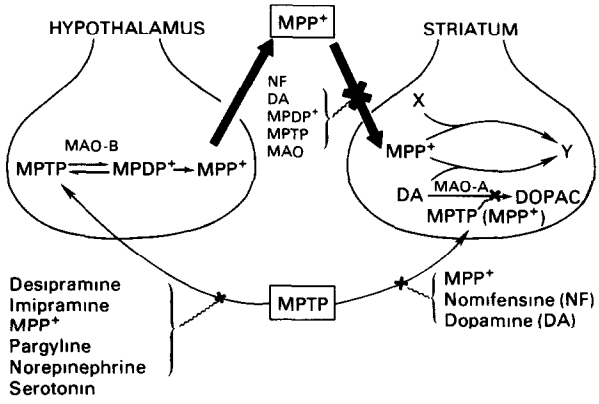


Fig.1. Hypothetical mechanism for serotonergic conversion of MPTP and dopaminergic accumulation of MPP$^{+}$. MPTP is accumulated into hypothalamic and striatal neurons at equal rates. Once inside the serotonergic neurons, MPTP is oxidized by MAO B to MPP$^{+}$, which may be released into interneuronal spaces. MPP$^{+}$ is then specifically accumulated into dopaminergic neurons in striatum, where it induces neurodegeneration. At the same time, the MPTP and MPP$^{+}$ which accumulate in the striatum could inhibit MAO A, elevating dopamine to neurotoxic levels.

ipramine, norepinephrine, and serotonin are potent inhibitors of this process. Since hypothalamus is rich in serotonergic neurons or terminals [27,28] which contain MAO B [29,30], MPTP may be converted to $MPP^+$ via $MPDP^+$ in these neurons.

$MPP^+$ (or $MPDP^+$) may be released from serotonergic neurons and/or astrocytes into the interneuronal spaces, which then carry it to dopaminergic neurons. The hypothalamic release of [$^3$H]$MPP^+$ may be a reversal of its uptake. If so, this process is approx. 3-fold faster than the uptake of [$^3$H]MPTP and may be equivalent to 43% of the uptake of norepinephrine or 75% of the uptake of serotonin. Such spontaneous release of $MPP^+$ from serotonergic neurons could explain their relative invulnerability to $MPP^+$ neurotoxicity.

Newly synthesized and released $MPP^+$ may then be specifically transported into the striatum. Since this process is selectively blocked by dopamine and nomifensine ($IC_{50}$ = 0.03–0.04 $\mu$M), this uptake may occur via the dopamine re-uptake system, as proposed by Javitch et al. [16], Sundstrom and Jonsson [14], and Uhl et al. [21]. Once in the striatum, $MPP^+$ (or $MPDP^+$) and MPTP could inhibit MAO A activity [31,32] and thus elevate dopamine to neurotoxic levels [33], although other toxic mechanisms could also account for the striatal cell destruction observed.

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## REFERENCES

[1] Davis, G.C., Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M. and Kopin, I.J. (1979) Psychiatr. Res. 1, 249–254.
[2] Langston, J.W., Ballard, P., Tetrud, J.W. and Irwin, I. (1983) Science 219, 979–980.
[3] Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M. and Kopin, I.J. (1983) Proc. Natl. Acad. Sci. USA 80, 4546–4550.
[4] Langston, J.W., Forno, L.S., Rebert, C.S. and Irwin, I. (1984) Brain Res. 292, 390–394.
[5] Markey, S.P., Johannessen, J.N., Chiueh, C.C., Burns, R.S. and Herkenham, M.A. (1984) Nature 311, 464–467.
[6] Langston, J.W., Irwin, I., Langston, E.B. and Forno, L.S. (1984) Neurosci. Lett. 48, 87–92.
[7] Chiba, K., Trevor, A. and Castagnoli, N. jr (1984) Biochem. Biophys. Res. Commun. 120, 574–578.
[8] Langston, J.W., Irwin, I., Langston, E.B. and Forno, L.S. (1984) Science 225, 1480–1482.
[9] Salach, J.I., Singer, T.P., Castagnoli, N. jr and Trevor, A. (1984) Biochem. Biophys. Res. Commun. 125, 831–835.
[10] Gessner, W., Brossi, A., Shen, R., Fritz, R.R. and Abell, C.W. (1984) Helv. Chim. Acta 67, 2037–2042.
[11] Fritz, R.R., Abell, C.W., Patel, N.T., Gessner, W. and Brossi, A. (1985) FEBS Lett. 186, 224–228.
[12] Mytilineou, C. and Cohen, G. (1984) Science 255, 529–531.
[13] Heikkila, R.E., Manzino, L., Cabbat, F.S. and Duvoisin, R.C. (1984) Nature 311, 467–469.
[14] Sundstrom, E. and Jonsson, G. (1985) Eur. J. Pharmacol. 110, 293–299.
[15] Cohen, G., Pasik, P., Cohen, B., Leist, A., Mytilineou, C. and Yahr, M.D. (1984) Eur. J. Pharmacol. 106, 209–210.
[16] Javitch, J.A., D'Amato, R.J., Strittmatter, S.M. and Snyder, S.H. (1985) Proc. Natl. Acad. Sci. USA 82, 2173–2177.
[17] Wieczorek, C.M., Parsons, B. and Rainbow, T.C. (1984) Eur. J. Pharmacol. 98, 453–454.
[18] Parsons, B. and Rainbow, T.C. (1984) Eur. J. Pharmacol. 102, 375–377.
[19] Javitch, J.A. and Snyder, S.H. (1984) Eur. J. Pharmacol. 106, 455–456.
[20] Irwin, I. and Langston, J.W. (1985) Life Sci. 36, 207–212.
[21] Uhl, G.R., Javitch, J.A. and Snyder, S.H. (1985) Lancet 1, 956–957.
[22] Gessner, W., Brossi, A., Shen, R. and Abell, C.W. (1985) J. Med. Chem. 28, 311–317.
[23] Gray, E.G. and Whittaker, V.P. (1962) J. Anat. 96, 79–87.
[24] Shen, R., Smith, R.V., Davis, P.J. and Abell, C.W. (1984) J. Biol. Chem. 259, 8994–9000.
[25] Gessner, W., Brossi, A., Shen, R. and Abell, C.W. (1985) FEBS Lett. 183, 345–348.
[26] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[27] Frankfurt, M., Lauder, J.M. and Azmitia, E.C. (1981) Neurosci. Lett. 24, 227–232.
[28] Steinbusch, H.W.M. and Nieuwenhuys, R. (1981) Adv. Exp. Med. Biol. 133, 7–35.

[29] Levitt, P., Pintar, J.E. and Breakefield, X.O. (1982) Proc. Natl. Acad. Sci. USA 79, 6385–6389.
[30] Westlund, K.N., Denney, R.M., Kochersperger, L.M., Rose, R.M. and Abell, C.W. (1985) Science, in press.
[31] Singer, T.P., Salach, J.I. and Crabtree, D. (1985) Biochem. Biophys. Res. Commun. 127, 707–712.
[32] Fuller, R.W. and Hemrick-Luecke, S.K. (1985) J. Pharmacol. Exp. Ther. 323, 696–701.
[33] Graham, D.G. (1984) Neurotoxicol. 5, 83–96.