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THE SPECIFIC VULNERABILITY OF THE SUBSTANTIA NIGRA TO MPTP IS RELATED TO THE PRESENCE OF TRANSITION METALS

Judes Poirier, John Donaldson, and André Barbeau

Department of Neurobiology, Clinical Research Institute of Montreal
Montreal, Quebec, Canada H2W 1R7

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SUMMARY: We demonstrate that the high concentration of transition metals in the substantia nigra could be a major factor responsible for the specificity of cell damage by the Parkinsonism-causing neurotoxin MPTP. It will be shown that these metals in vitro, and MPTP, each potentiate the autoxidation of dopamine and the production of aminochrome through the generation of superoxide, hydroxyl radicals, hydrogen peroxide and reactive semiquinones. Moreover, the same metals contribute to the oxidation of MPTP itself, further enhancing dopamine autoxidation.

Numerous studies on the newly discovered neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that it can cause Parkinsonism in man (1,2) and animals(3-8). MPTP binds with (9,10), and is metabolized by (11,12), monoamine oxidase (MAO), mostly but not only into the 1-methyl-4-phenyl pyridinium ion (MPP^+) (11,13-15). The toxicity of MPTP is blocked by MAO inhibitors in vitro (11,12,16) and in vivo (8,13,17,18). Unfortunately these investigations have failed to explain why the cytotoxicity of MPTP in the brain, probably related to the generation of reactive species (19), is localized almost exclusively to the substantia nigra (1,3,4,7,20). The pattern of MPTP cell damage is indeed different from the total distribution in the brain of MPP^+ (14), dopamine (19), MAO (10) or even neuromelanin (21), despite the affinity of MPTP to the latter two (10,22). Areas such as the locus coeruleus (3,4) and the mesolimbic dopamine system (20),

Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenyl pyridinium ion; DA, dopamine; MAO, monoamine oxidase; $SQ^{\cdot}$, dopamine semiquinone reactive species; Q, dopamine quinone; $X^{\cdot}$, MPTP reactive species; $O_2^{\cdot-}$, superoxide; $OH^{\cdot}$, hydroxyl radical; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; OH^-, hydroxyl ion; SeGSH-Px, selenium glutathione peroxidase.

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sharing many of the same components as the substantia nigra, are apparently spared. Finally, cell damage caused by MPTP is not correlated to sites of normal antioxidant activity, since superoxide dismutase (SOD) is in high concentration in the brain while catalase and glutathione peroxidase are in low levels except in the substantia nigra (23). Donaldson et al (25) have shown that manganese neurotoxicity could be due to the potentiation by that metal of dopamine autoxidation, thereby explaining some of the extrapyramidal symptoms produced (31). In the present paper we will demonstrate that the high concentration of trace metals in the substantia nigra (24), in combination with the other components referred-to above, could confer to that nucleus its specific vulnerability to MPTP.

METHODS

The rate of autoxidation of dopamine was determined by measuring the increase in absorbance of aminochrome formation (at 480 nm), in the presence and absence of metal ions, MPTP, MPP^+ or Paraquat, using a Beckman Acta M-VI spectrophotometer equiped with kinetic accessories, according to the method of Donaldson et al (25). Experiments with metals were performed in vitro using 5 ml of 0.1 M concentration of phosphate buffer at pH 7.8 with double-distilled deionised water. Dopamine HCl (Calbiochem) was employed at the concentration of 500 µM. The metals were in freshly prepared solutions at the fixed concentration of 10 µM. Incubation was carried out at 37°C in a shaking water bath. MPTP (Aldrich), MPP^+ (gift of Dr. J.W. Langston) and Paraquat (Sigma), were in solution at the concentration of 35 µM. The concentrated enzymes catalase and superoxide dismutase (Sigma) were used at various concentrations. The metal chelator EDTA was used at 10^{-3} M. The chemical reactions involved in the various experimental situations are listed in Fig. 1.

RESULTS AND DISCUSSION

(1) Reaction between MPTP and transition metals: The oxidation of divalent metals produces free radicals (26) (reactions 1-4). We prepared fresh aqueous solutions of MPTP and various divalent metals and followed the absorption at 500 nm. MPTP was extremely sensitive to divalent metals and readily oxidized to form insoluble hydroxylated co-derivatives of characteristic colors, as confirmed by control solutions of the metals in NaOH. Generally, the $metal^{+3}$ precipitate appeared first but some 48 hours later both the $metal^{+3}$ and $metal^{+2}$ precipitates were recognized.

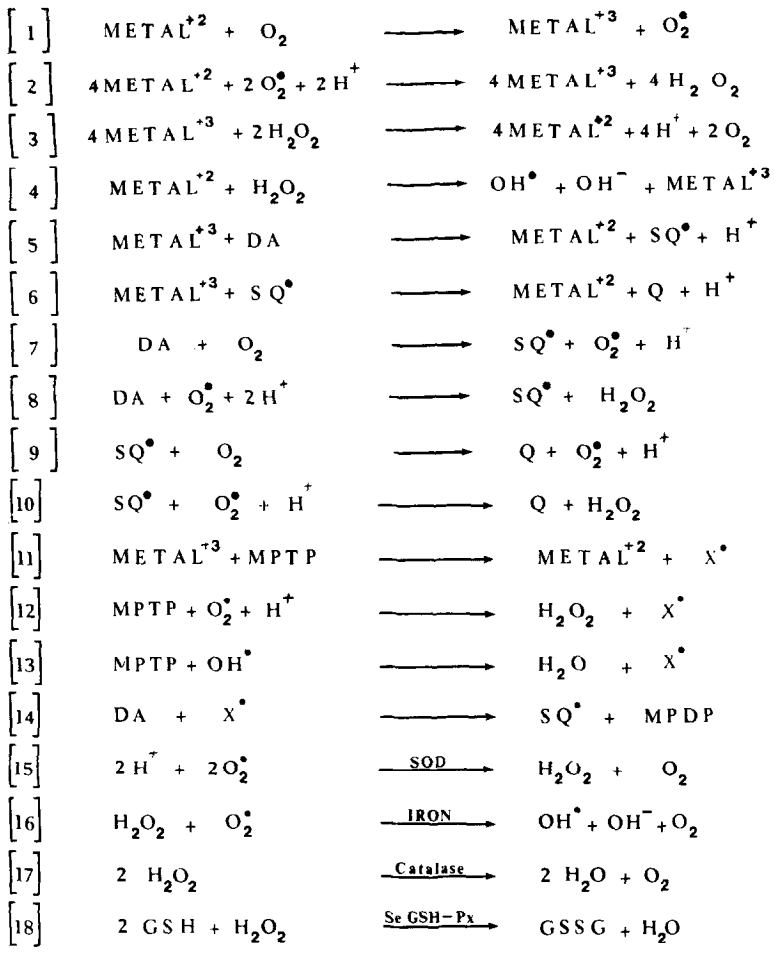

Fig. 1. Chemical reactions possibly involved in the potentiation by transition metals of MPTP-induced autoxidation of dopamine.

Chelation of hydroxyl ions by trivalent metals was shown to decrease the pH of the solution in a dose-dependent manner related to the production of the precipitates. The interaction of MPTP and divalent metals in aqueous solution therefore implicates electron transfer reactions with the formation of a new MPTP reactive species (which we call: X$^{\bullet}$) plus trivalent metals (reactions 1 and 12). Under the same conditions MPP^+ and Paraquat do not cause similar precipitates.

To prove the presence of superoxide, we added SOD to the solution. SOD, which normally transforms $O_2^{\bullet-}$ into H_2O_2 by dismutation (reaction 15), did indeed quantitatively decrease the formation of the ferric hydroxyde precipitate (Fig. 2 A). The most likely mechanism for this is that MPTP and

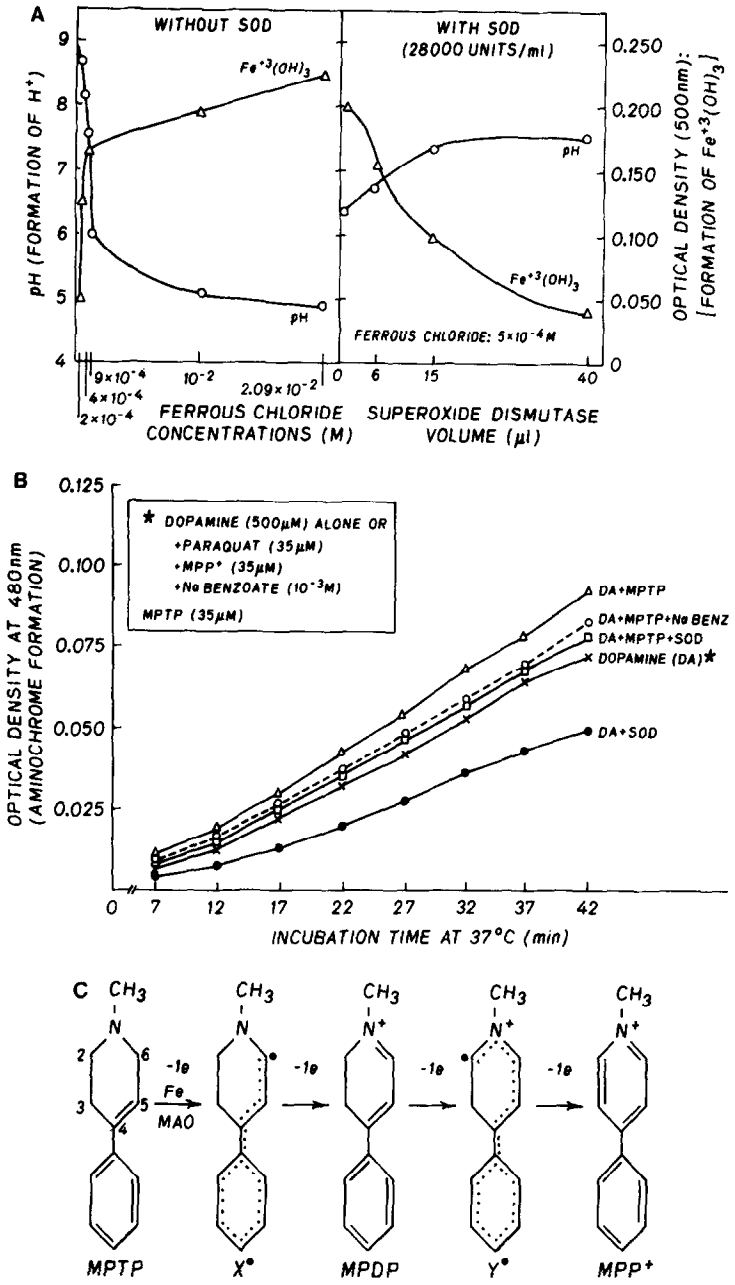

Fig. 2. (A) Oxidation of ferrous chloride in the presence of MPTP (10^{-3} M) with or without various volumes of superoxide dismutase (28,000 units/ml). Concentrations of ferrous chloride used were 2 x 10^{-4} M, 4 x 10^{-4} M, 10^{-2} M and 2.09 x 10^{-2} M. With SOD, ferrous chloride concentration was 5 x 10^{-4} M. (B) Modification of dopamine autoxidation by MPTP (35 µM), MPP^+ (35 µM), Paraquat (35 µM), SOD (50 µg/5 ml) and sodium benzoate (10^{-3} M). (C) Proposed metabolism of MPTP. (D) Effect of various doses of catalase (from 0 to 12,000 units/5 ml) on dopamine autoxidation potentiated by MPTP. The insert indicates the percentage decrease in aminochrome formation by the addition of catalase. Incubation time is 37 minutes at 37°C. (E) Effect of Fe^{+2} (10 µM) and MPTP (35 µM), alone or in combination upon dopamine (500 µM) autoxidation. Incubation time: 57 minutes at 37°C. (F) Similar conditions to (D) with Mn^{+2} (10 µM) being used.

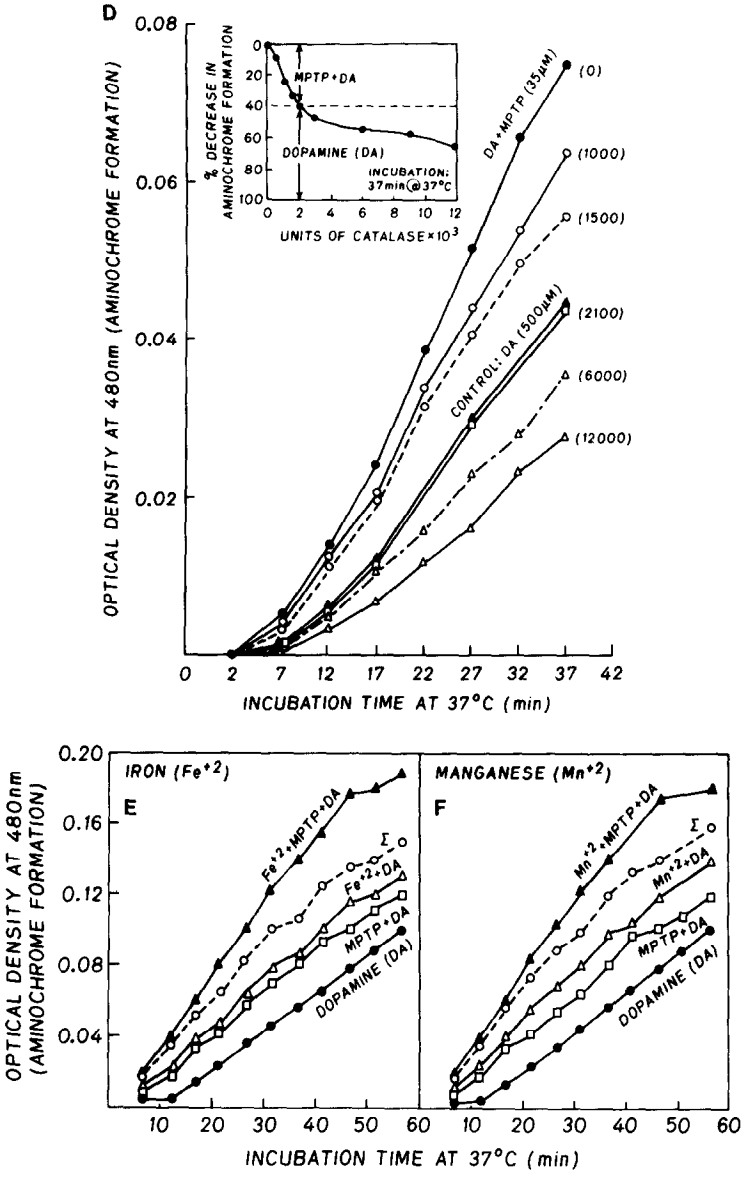

Fig. 2 -- continued

SOD both compete for $O_2^{\cdot}$ in the generation of H_2O_2 and of the MPTP reactive species X$^{\cdot}$ (reaction 12). Finally, the production of the hydroxyl radical (OH$^{\cdot}$) and of the hydroxyl ion (OH^-) probably occurs through reactions 4 or 16, thus explaining the formation of the hydroxylated derivatives described above. MPTP can interact with OH$^{\cdot}$ to again generate the new reactive species X$^{\cdot}$ (reaction 13).

(2) Potentiation of dopamine autoxidation by divalent metals: Dopamine, like its 6-hydroxy derivative, can autoxidate (27-29). The main products are hydrogen peroxide and dopamine orthoquinone, with superoxide serving as a chain-propagating radical in the autoxidation of dopamine and other polyphenols (30). The addition of divalent metals to dopamine, under the conditions described above, potentiated the autoxidation through the production of the strong oxidants: trivalent metals and more superoxide (reactions 1 and 5-10). Our own experiments (Table 1) demonstrate for the first time that iron is almost as powerful as manganese in that system (25).

(3) Potentiation of dopamine autoxidation by MPTP: In contrast to MPP^+ and to the potent herbicide Paraquat which are inactive in our system, MPTP potentiates the autoxidation of dopamine in vitro, as shown in Fig. 2 B. The formation of aminochrome, in the presence of fixed amounts of MPTP, was directly correlated to the concentration of dopamine used. In vivo, the oxidation of MPTP is initiated by the enzyme MAO and other monooxygenases and probably involves reactive species ($O_2^{\cdot}$ and $OH^{\cdot}$), hydrogen peroxide, and possibly transition metals (13-18). In vitro, the presence of the same substances was demonstrated through the following experiments:

(a) The addition of SOD (50 µg/5 ml) decreased dopamine autoxidation by 32 %, but the MPTP potentiation of this autoxidation by 71 % (Fig. 2 B), thus proving the presence of superoxide in the solution.

Table 1
Potentiation by metals of dopamine and MPTP-induced dopamine autoxidation (incubation time: 57 minutes)

Solutions	Dopamine autoxidation O.D. at 480 nm	% Potentiation over MPTP-induced DA autoxidation
1. Dopamine (DA) alone	0.100	-
2. DA + MPTP	0.120	-
3. DA + $Calcium^{+2}$	0.087	0
4. DA + $Zinc^{+2}$	0.090	0
5. DA + $Cobalt^{+2}$	0.112	31 %
6. DA + $Iron^{+2}$	0.133	53 %
7. DA + $Manganese^{+2}$	0.141	59 %

(b) The addition of only 2,100 units of catalase reduced the MPTP-induced potentiation of dopamine autoxidation by 97 % (Fig. 2 D), indicating that this potentiation involves mainly the formation of hydrogen peroxide from superoxide and/or MPTP (reactions 2,8,12 and 15). In fact, 40 % of the total generation of aminochrome, mostly the part due to the dopamine-MPTP interaction, is inhibited by as little as 2,100 units of catalase (Fig. 2 D - insert). A further inhibition of 25 % in the formation of aminochrome requires as much as 10,000 more units of catalase.

(c) The addition of sodium benzoate (10^{-3} M), which chelates hydroxyl radicals (OH'), had no effect on the autoxidation of dopamine itself, as previously shown by Cohen and Heikkila (28). However, the same amount of sodium benzoate inhibited 61 % of the potentiation of dopamine autoxidation induced by MPTP (Fig. 2 B). Hydroxyl radicals must therefore be generated during the MPTP-dopamine interaction, probably by the metal catalysed Haber-Weiss equation (reaction 16).

(d) The addition in vitro of EDTA (10^{-3} M), inhibits only 17 % of the autoxidation of dopamine, but completely blocks (100 %) the potentiation of that autoxidation by MPTP. Since no metals were consciously added to the solution, trace amounts of a transition metal must have been present in a quantity sufficient to initiate the oxidation of dopamine, and mainly to potentiate the oxidation of dopamine by MPTP (reactions 1,5 and 12 to 14).

(4) Potentiation of MPTP-induced enhancement of dopamine autoxidation by transition metals: In other experiments described in Table 1, cobalt, manganese and iron clearly potentiate the MPTP-induced enhancement of dopamine autoxidation, which itself represented a 20 % potentiation over the baseline autoxidation of dopamine. Zinc and calcium, however, were inactive. This effect is clearly illustrated in Fig. 2 E and F for iron and manganese, in the presence of a fixed amount of MPTP (35 uM). The resulting potentiation of dopamine autoxidation far exceeds that induced by MPTP or by either of the metals alone. In fact, this metal-plus MPTP-induced potentiation exceeds the sum of the two independent effects. These results indicate that

transition metals help initiate and propagate the autoxidation of dopamine (reaction 1 and 5-10), while at the same time oxidizing MPTP itself into the reactive species X· (reactions 1,12,13). X· and co-products in turn (reactions 14 and 16) accelerate the autoxidation of dopamine.

(5) Reactive species of MPTP and MPTP metabolism: The successive loses of one electron from MPTP will eventually produce 1-methyl-4-phenyl-pyridinium ion (MPP^+) (13,14) as seen in Fig. 2 C. The first step leads to the reactive species which we have called X·, (illustrated in the figure in its resonance form which should confer to it a greater stability). The important moment in the toxicity of MPTP is thus the generation of this X·, a reaction which necessitates the presence of the C_4-C_5 double-bound (32). Subsequently, X· will lose the free electron to form the intermediate MPDP (11). This electron can easily be transferred to dopamine (reaction 14), to cytochromes, or to oxygen, again forming superoxide, thus further accelerating dopamine's autoxidation process. These experiments do not indicate that the generation of free radicals is the cytotoxic mechanism, but only that metals promote the formation of aminochrome (a well known precursor of neuromelanin) by MPTP through the mechanism of free radical production. In fact, evidence from other experiments (Donaldson, Poirier and Barbeau, unpublished) indicate that induced lipid peroxidation is inhibited by MPTP in brain and liver homogenates, suggesting that the neuromelanin pathway may be more important to the mechanism of MPTP toxicity and, by extension, to the cell death process in Parkinson's disease.

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