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Neurotoxicity, Drugs of Abuse, and the CuZn-Superoxide Dismutase Transgenic Mice

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Abstract

Administration of methamphetamine (METH) to animals causes loss of DA terminals in the brain. The manner by which METH causes these changes in neurotoxicity is not known. We have tested the effects of this drug in copper/zinc (CuZn)-superoxide dismutase transgenic (SOD Tg) mice, which express the human CuZnSOD gene. In nontransgenic (non-Tg) mice, acute METH administration causes significant decreases in DA and dihydroxyphenylacetic acid (DOPAC) in the striata of non-Tg mice. In contrast, there were no significant decreases in striatal DA in the SOD Tg mice. The effects of METH on DOPAC were also attenuated in SOD Tg mice. Chronic METH administration caused decreases in striatal DA and DOPAC in the non-Tg mice, but not in the SOD-Tg mice. Similar studies were carried out with 1-methyl-1,2,3,6-tetrahydropyridine (MPTP), which also causes striatal DA and DOPAC depletion. As in the case of METH, MPTP causes marked depletion of DA and DOPAC in the non-Tg mice, but not in the SOD Tg mice. These results suggest that the mechanisms of toxicity of both METH and MPTP involve superoxide radical formation.

Index Entries: Transgenic mice; superoxide dismutase; methamphetamine; MPTP; neurotoxicity; free radicals; striatum; dopamine.

Introduction

Oxidative mechanisms have now been implicated in a number of pathological states that affect both the central and the peripheral nervous systems. These include phenomena associated with the aging process (Ames et al., 1993), other neurodegenerative phenomena, such as Parkinson's disease (Cadet, 1988), amyotrophic lateral sclerosis (Deng et al., 1993;

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Rosen et al., 1993), neurovascular accidents (Hall and Braughler, 1989; Chan et al., 1990, 1991), as well as neurotoxic effects of a number of drugs. More recently, neurotoxic phenomena that were thought to be only the result of excitotoxicity have now been shown to involve oxidative stress as a final common pathway to cell death (Dawson et al., 1993; *see also* review by Coyle and Puttfarcken, 1993). Cadet (1988) had earlier discussed the possibility that some neuronal cells might be especially sensitive to the effects of increased oxygen-based radicals derived during states that cause increased dopamine metabolism.

In spite of the active interest in the possible role of free radicals in central mechanisms, it has been difficult to study these processes directly. More recently, using transgenic animal technology, it has become possible to increase the level of specific proteins, such as the copper/zinc superoxide dismutase (CuZnSOD) enzyme, that are involved in the breakdown of oxygen-derived free radicals (Epstein et al., 1987). SOD enzymes are involved in the dismutation of the superoxide radical, O_2, to H_2O_2, which is then metabolized to water by glutathione peroxidase or catalase. Although these enzymes are thought mainly to be protective, it is very important to point out that they can also participate in the generation of toxic substances. Specifically, H_2O_2 produced as a result of SOD activity may, in combination with the superoxide radical or in the presence of some metals, give rise to the very reactive hydroxyl radical (OH). Thus, because of our interest in the role of free radicals in DA and DA-adjacent systems, we have conducted a series of studies in which we have evaluated the effects of increased CuZnSOD gene dosage on neuroreceptors in the CuZnSOD Tg mice (Cadet et al., 1990, 1993; Kujirai et al., 1994). We have also conducted some studies investigating the role of the superoxide radicals in the neurotoxic effects of methamphetamine (Cadet et al., 1994) and MPTP (Przedborski et al., 1992) on the DA systems of these mice. The purpose of the present article is to provide both a review of those studies and to provide models that take into consideration some of the manipulations that have been shown to be protective against these two agents.

Effects of Methamphetamine Administration to CuZnSOD TG Mice

Methamphetamine (METH) is a drug of abuse that is a known neurotoxicant (Seiden et al., 1975; Kogan et al., 1976; Ricaurte et al., 1980; Bakhit et al., 1981; Marek et al., 1990). Specifically, METH administration causes significant decreases in striatal dopamine (DA) and serotonin (5-HT) levels, changes that are associated with loss of DA and 5-HT terminals in rats and mice (Kovachich et al., 1989; Ali et al., 1994b). Because intact DA systems are necessary for the manifestations of these changes (Gibb and Kogan, 1979; Seiden and Vosmer, 1984; Schmidt et al., 1985), oxidative stress is thought to play a role in these changes (De Vito and Wagner, 1989). Recent studies have, nevertheless, hinted at the possibility that excitatory amino acids might also be involved in the neurotoxic effects of METH, because glutamate receptor antagonists attenuate the abnormalities observed in the nigrostriatal DA system (Sonsalla et al., 1989). Altogether, these reports support a role for both excitotoxins and free radicals in causing DA after METH administration. In order to test these ideas further, we have made use of the CuZnSOD Tg mouse model in order to determine if oxidative stress is an important factor in the causation of METH-induced neurotoxic effects on monoaminergic systems.

Both acute and chronic studies were carried out. In the acute experiments, mice were given either saline or a large dose (25 mg/kg, subcutaneously [sc]) of d-METH. Mice were sacrificed 6 h later. In the chronic experiments, the mice were first given two daily injections (bid) of METH (5 mg/kg, sc) for 6 d.

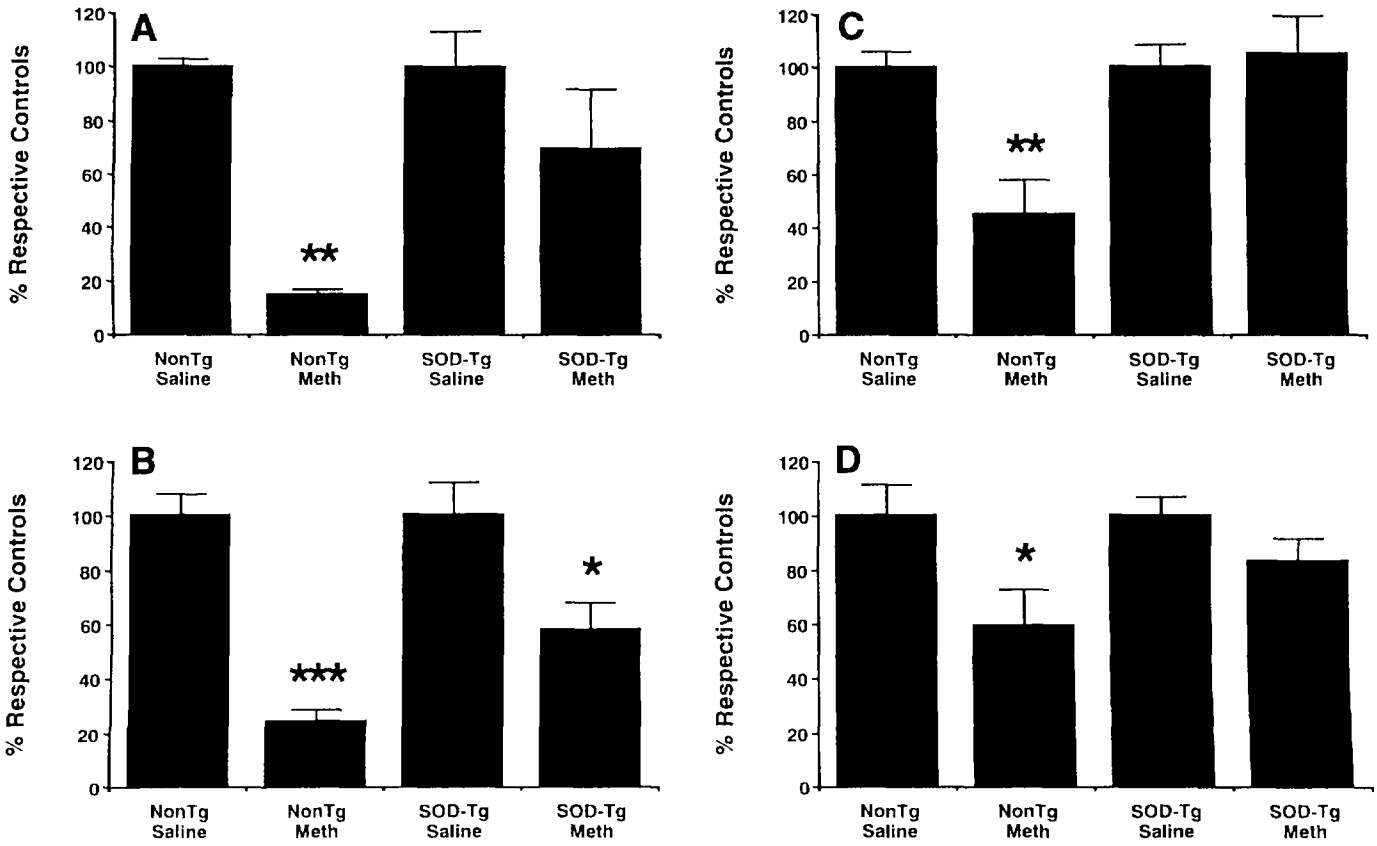

Fig. 1. Effects of acute **(A,B)** and chronic **(C,D)** administration on striatal dopamine and DOPAC levels in non-Tg and CuZnSOD Tg mice. Acute administration of METH caused significant decreases in DA levels in only the non-Tg mice, whereas DOPAC levels were affected in both groups of mice, although the percent decrease was smaller in the SOD Tg (–42%) in comparison with the non-Tg (–76%) mice. Chronic administration of METH significantly decreased striatal DA and DOPAC in only the non-Tg mice. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, in comparison with saline-treated animals (Modified from Cadet et al., 1994).

The injections were increased to METH (10 mg/kg, bid) for 1 d. Then, these were increased to METH (15 mg/kg, sc, bid) for another day. The animals were sacrificed 1 wk after the last injection.

The effects of METH administration on DA and dihydroxyphenylacetic acid are shown in Fig. 1. Acute administration of METH caused significant changes in DA levels in the striatum (Fig. 1A). These changes were the result of decreases in DA in the control mice, whereas the SOD-Tg mice were not significantly affected. DOPAC levels were decreased in both the SOD Tg and in the non-Tg mice, with the percentage of decreases being greater in the non-Tg (–76%) than in the SOD Tg (–42%) (Fig. 1B). Chronic administration of METH causes significant changes in DA levels in the striatum (Fig. 1C). These were owing to decreases in DA caused by METH in the nontransgenic animals. The SOD Tg mice were not affected. DOPAC levels were also decreased in the non-Tg mice only (Fig. 1D).

Thus, both acute and chronic administration of METH caused marked decreased in DA levels mainly in the non-Tg mice. These results do support the view that oxidative stress is an important mechanistic event in the develop-

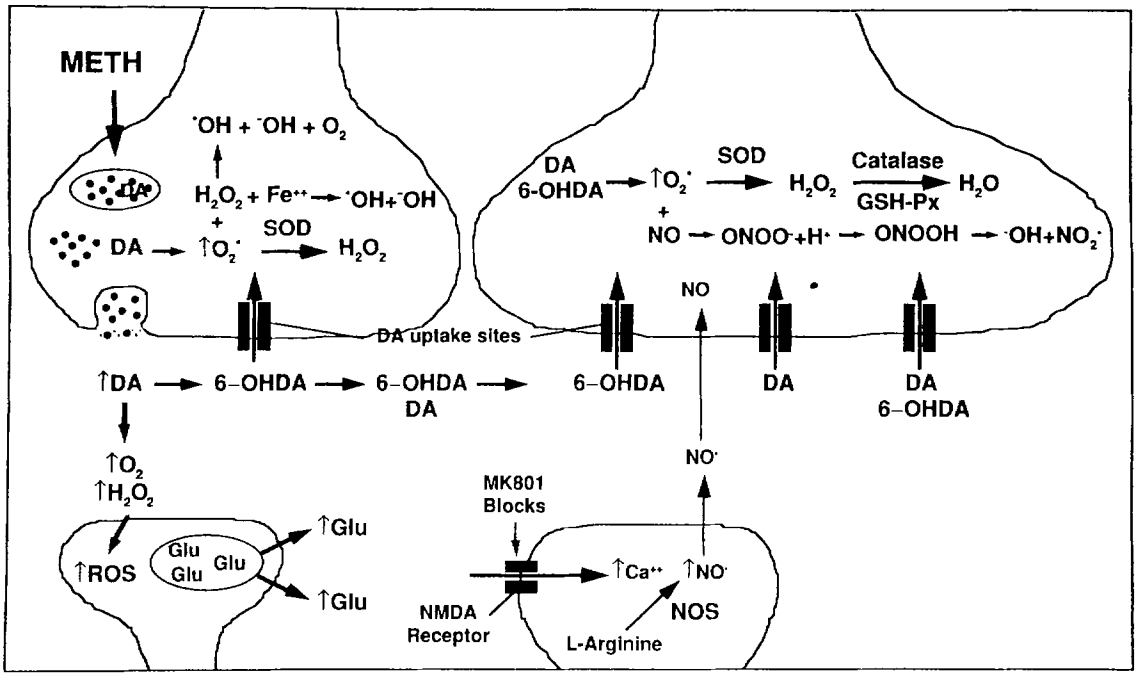

Fig. 2. Possible involvement of the superoxide radical in the neurotoxic effects of METH. This figure takes into consideration some of the manipulations that have been shown to protect against the neurotoxic effects of the drug. *See text* for more details.

ment of these changes (Seiden and Vosmer, 1984; De Vito and Wagner, 1989). Other investigators have made similar arguments based on the attenuating effects of manipulating DA synthesis (Gibb and Kogan, 1979). The partial protection observed in DOPAC levels in the SOD Tg mice suggests that a large dose of METH might be able to partially overwhelm the scavenging systems of the SOD Tg mice, despite the high levels of CuZnSOD in these animals. This statement also suggests that chronic METH administration may result in the formation of enough 6-hydroxydopamine (6-OHDA) (Seiden and Vosmer, 1984) and associated ROS (Cohen and Heikkila, 1974) in the striatum, with consequent DA and DOPAC depletion observed only in the non-Tg mice because the SOD Tg mice are much better able to ward off the progressive damage caused by the more protracted increase in oxygen-based radicals. Because glutamate receptor blockers also attenuate METH-induced toxicity (Sonsalla et al., 1989), it could be argued that release of excitatory amino acids must also play a role in the manifestations of the deleterious effects of this drug. This argument does not deter from the present results, but hints at the notion that free radical-induced changes may constitute a common pathway for the causation of damage in the central nervous system (*see* Coyle and Puttfarcken, 1993; Dawson et al., 1993). The recent report that stimulation of glutamate receptors generates the superoxide radical supports this argument (Lafon-Cazal et al., 1993).

Figure 2 provides a model that takes into consideration both the involvement of free radicals and of excitotoxins in METH-induced DA terminal loss. Thus, the acute release of DA caused by METH may be associated with increased formation of 6-OHDA both within and outside of the DA terminals. 6-OHDA

is then retaken up by the DA terminals. Superoxide radicals and hydrogen peroxide produced during the metabolism of either DA or 6-OHDA may be partially responsible for DA terminal loss caused by METH administration. Stimulation of glutamate receptors may result in the formation of nitric oxide (NO), which might be taken up by the DA terminals. NO itself might be toxic to DA neurons; moreover, within DA neurons, NO might interact with the superoxide radical to make peroxynitrite and, subsequently, the very toxic hydroxyl radical (Moncada et al., 1991). It should be pointed out, however, that even in the absence of 6-OHDA, the production of cytotoxic quinones within the DA terminals might also lead to their demise.

Effects of MPTP Administration to CuZnSOD TG Mice

1-Methyl-4-phenyl,1,2,3,6-tetrahydropyridine (MPTP) causes irreversible parkinsonism in both human and nonhuman primates (Burns et al., 1983; Langston et al., 1983). These parkinsonian signs and symptoms are associated with marked depletion of DA and its metabolite in the striatum. After peripheral administration, MPTP has also been shown to cause marked depletion of DA and of its metabolites in mice (Heikkila et al., 1984). Although the neurotoxic effects of MPTP have been attributed to the inhibition of the mitochondria electron transport chain by the *N*-methyl-4-phenylpyridium ion (MPP^+), which results in the biotransformation of MPTP by monoamine oxidase B (MAOB) (Heikkila et al., 1985) and subsequent ATP depletion (Schotcher et al., 1990), the possibility still existed that oxygen-based radicals produced either by the MAOB-catalyzed biotransformation step, by MPP^+-induced blockade of Complex I (Hasegawa et al., 1990) or by redox cycling reactions (Adams et al, 1993; Klaidman et al., 1993), may also have participated in killing DA neurons. Recently, Ali et al. (1994a) reported the generation of reactive oxygen species after acute administration of MPTP.

We thus used the SOD Tg mice to test the hypothesis that increased production of superoxide radicals might be involved in the neurotoxic effects observed in the DA systems after ip injection of MPTP-HCl. Both subacute and chronic studies were carried out in both types of mice. In the subacute studies, the mice received three daily injections of MPTP (30 mg/kg) and were sacrificed 5 d after the last injection. In the chronic studies, the mice received similar doses of MPTP, except that they were sacrificed 3 wk later.

Figure 3 shows the results of the MPTP injections. Administration of MPTP to non-Tg albino mice caused marked decreases in DA and DOPAC levels, [^{3}H]mazindol-labeled DA uptake sites, and decreased [^{3}H]DA uptake in striatal synaptosomes (Przedborski et al., 1992). The neurotoxic effects of MPTP were not observed in the SOD Tg mice.

It appears, therefore, that superoxide radicals are in fact involved in the pathogenesis of the toxic effects of MPTP on dopaminergic cells. Figure 4 provides a working model for the involvement of the superoxide radical in the neurotoxicity of MPTP. This model is in agreement with recent data, which show that the peripheral administration of MPTP or the use of its metabolites in vitro does cause oxidative stress (Johannessen et al., 1986; Chieuh et al., 1993; Adams et al., 1993; Klaidman et al., 1993; Ali et al., 1994a). Thus, after entry into the brain, MPTP is metabolized by MAOB in astrocytes to make MPP^+ (*see* Johannessen, 1991 and Adams and Odunze, 1991 for further details). MPP^+ is then taken up by DA uptake sites into DA terminals, where it accumulates within mitochondria. Within the mitochondria, MPP^+ blocks Complex I. The blockade of Complex I results in both the production of superoxide radicals and in energy loss within the DA terminals; both of these mechanisms may play important roles in the cell death observed after MPTP administration.

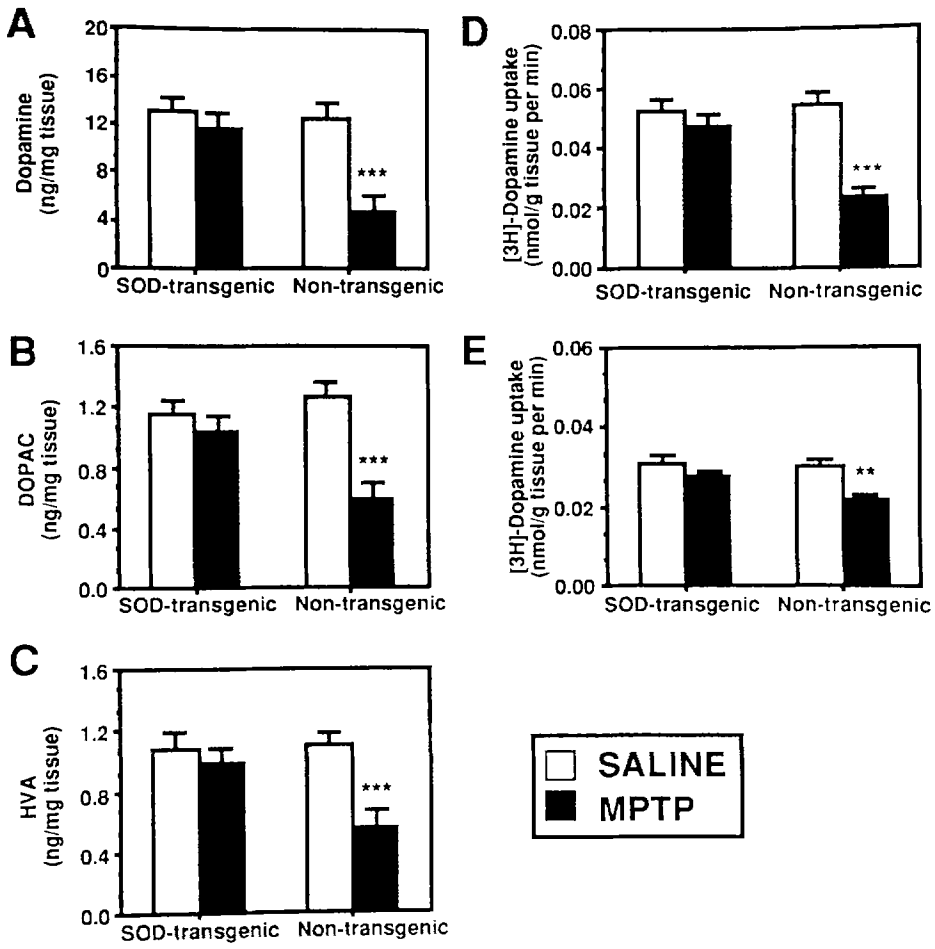

Fig. 3. Effects of MPTP on the concentration of dopamine (**A**), DOPAC (**B**), HVA (**C**), ^{3}H-DA in the striatum (**D**), and substantia nigra (**E**) of SOD TG and their non-TG littermates after MPTP injections. MPTP causes significant decreases in DA, DOPAC, HVA, and ^{3}H-DA uptake in the striatum and the substantia nigra of non-TG mice, but does not affect these parameters in SOD TG mice. Each value represents mean + SEM of 5 animals/group. $^{**}p < 0.01$; $^{***}p < 0.001$ differences between MPTP-treated non-TG mice and all other groups (modified from Przedborski et al., 1992).

Conclusions

These studies are of both basic and clinical importance. For instance, CuZnSOD enzyme has been suggested as a therapeutic agent for a number of conditions in which free radical mechanisms have been implicated. These include ischemia and reperfusion, as well as other forms of perturbations (Chan et al., 1987; Cohen, 1989; Jackson et al., 1990; Das et al., 1991; Hirsch et al., 1992). Although it is not yet possible to state categorically the importance of increased CuZnSOD activity in the brain (Warner, 1994), the present data indicate that the neurotoxicity of agents that affect the DA systems involves the increased generation of superoxide radicals. When taken together, these observations support the contention that oxygen-based radicals may form parts of the deleterious substrates that lead to the manifestations of a number of pathobiological entities (Cadet, 1988).

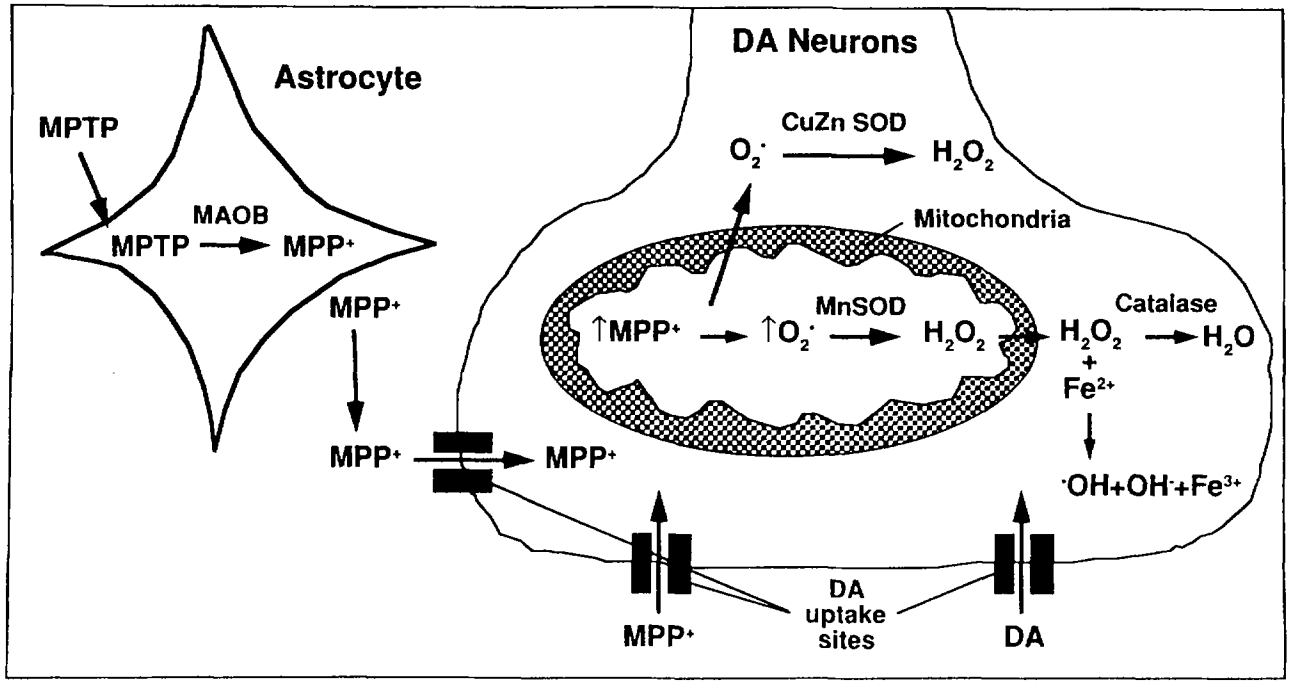

Fig. 4. Possible involvement of superoxide radicals in the neurotoxic effects of MPTP. The figure takes into consideration some of the manipulations that have been shown to attenuate the toxic effects of the drug. It also takes into consideration the metabolic pathway of the drug.

Acknowledgments

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