0026-895X/96/061511-09$3.00/0

MOLECULAR PHARMACOLOGY, 50:1511–1519 (1996).

Transgenic Mice with High Levels of Superoxide Dismutase Activity are Protected from the Neurotoxic Effects of 2′-NH_2-MPTP on Serotonergic and Noradrenergic Nerve Terminals

ANNE M. ANDREWS, BRUCE LADENHEIM, CHARLES J. EPSTEIN, JEAN LUD CADET, and DENNIS L. MURPHY

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20892 (A.M.A., D.L.M.), Molecular Neuropsychiatry Section, National Institute on Drug Abuse, Baltimore, Maryland 21224 (B.L., J.L.C.), and Department of Pediatrics, University of California, San Francisco, California 94143 (C.J.E.).

Received April 12, 1996; Accepted September 10, 1996

SUMMARY

Administration of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analog 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP; 4 × 15 mg/kg) to CD-1 mice was found to cause substantial decreases in cortical and hippocampal 5-hydroxytryptamine (5-HT) and norepinephrine (NE) to 20–30% of control 3 weeks after treatment. The magnitude of these depletions was similar to those reported previously in Swiss Webster and C57BL/6 mice given 4 × 20 mg/kg 2′-NH_2-MPTP, and in keeping with these prior studies, striatal dopamine levels were unchanged by 2′-NH_2-MPTP treatment in CD-1 mice. Subsequently, transgenic CD-1 mice producing high levels of human cytosolic Cu-Zn superoxide dismutase (SOD) were studied to assess the role of oxygen radicals in the mechanism of action of 2′-NH_2-MPTP. In contrast to the results described above, 5-HT and NE levels were almost completely unaffected by 2′-NH_2-MPTP treatment in homozygous SOD mice bearing 5-fold increases in brain SOD activity. In 2′-NH_2-MPTP-treated heterozygous SOD mice, which showed an average 3-fold increase in brain SOD activity, only moderate depletions in cortical and hippocampal 5-HT (50–60% of control) and NE (30–40% of control) were observed. Additionally, the density of [^{125}I]RTI-55-labeled 5-HT uptake sites was studied to further assess possible 5-HT terminal loss. In various cortical and hippocampal subregions of nontransgenic mice, 5-HT uptake sites were reduced to 20–35% of control after 2′-NH_2-MPTP treatment, in comparison with homozygous SOD mice, which were affected only minimally by 2′-NH_2-MPTP administration, and heterozygous SOD mice, which showed intermediate reductions in 5-HT uptake site density on the order of 55–80% of control. Together, these data indicate that mice genetically endowed with increased SOD activity are protected from 2′-NH_2-MPTP-induced toxicity, thereby implicating superoxide radicals in the mechanism of action of a neurotoxin that selectively depletes 5-HT and NE without affecting dopamine.

MPTP is a dopaminergic neurotoxin in humans (1, 2), nonhuman primates (3, 4), and mice (5, 6) that causes nigrostriatal neurodegeneration that is strikingly similar to idiopathic Parkinson's disease. For this reason, much research has been directed at the elucidation of MPTP's neurotoxic mechanism of action, and many analogs of MPTP have been synthesized and subsequently tested for their ability to deplete striatal DA as part of structure-activity studies (7–19). Most analogs that bear a substituent at the 2′ position have been found to be potent DA depletors (Fig. 1); however, studies of 2′-NH_2-MPTP (Fig. 1), a 2′-amino-substituted analog of MPTP, show that this analog is unique in that it preferentially depletes 5-HT (serotonin) and NE in mouse brain, primarily in the frontal cortex and hippocampus, without affecting striatal DA (20–22).

Although the target neuronal subpopulations of their toxicity are different, the initial steps in the mechanism of action of MPTP versus 2′-NH_2-MPTP are similar in some respects. MPTP-induced neurotoxicity requires oxidation by MAO-B (23, 24), and its DA-depleting effects are prevented by inhibition of DA uptake (25). In the case of 2′-NH_2-MPTP, neurotoxicity is prevented by inhibition of MAO-A (21), and

This work was supported by the Intramural Research Programs of the National Institute of Mental Health and National Institute on Drug Abuse. A.M.A. was supported by an Intramural Research Training Award from National Institute of Mental Health.

ABBREVIATIONS: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; HPLC, high performance liquid chromatography; HVA, homovanillic acid; RTI-55, 3β-(4′-iodophenyl)tropan-2β-carboxylic acid methyl ester; RTI-121, 3β-(4-iodophenyl)-tropane-2-carboxylic acid isopropyl ester; MAO, monoamine oxidase; MDMA, 3,4-methylenedioxymethamphetamine; NE, norepinephrine; 2′-NH_2-MPTP, 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine; non-Tg, nontransgenic CD-1 mice; SOD, Cu-Zn superoxide dismutase; SOD-heteroTg, heterozygous SOD transgenic; SOD-homoTg, homozygous SOD transgenic.

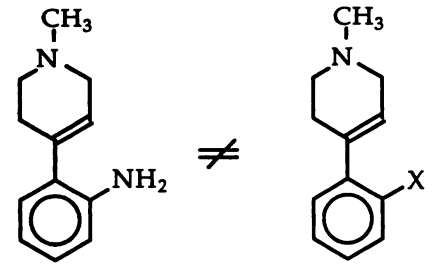

Fig. 1. Structure of 2'-NH_2-MPTP versus other related MPTP analogs. 2'-NH_2-MPTP selectively depletes 5-HT and NE in cortex and hippocampus without affecting striatal DA. By contrast, other structurally similar MPTP analogs with various substituents in the 2' position have been found to be potent striatal DA depletors.

2'-NH_2-MPTP-induced serotonergic and noradrenergic depletions are attenuated selectively by pretreatment with 5-HT or NE uptake inhibitors, respectively (21, 22). These results imply that 2'-NH_2-MPTP or, more likely, an MAO-derived metabolite, exerts its toxic effects after accumulation in 5-HT and NE nerve terminals via their respective transporters.

Further studies on the mechanism of action of MPTP suggested that oxygen-based free radicals may play an important role in MPTP's neurodegenerative effects in the nigrostriatal DA system (26–29). However, there is little evidence in the literature on the role of oxyradicals with regard to neurotoxicity in the serotonergic and noradrenergic neurotransmitter systems. Because 2'-NH_2-MPTP is the only neurotoxin currently known that selectively depletes 5-HT and NE in the mouse without decreasing DA after systemic administration, 2'-NH_2-MPTP seems to be ideally suited for the broader study of the contribution of free radicals to neurotoxicity in monoaminergic systems other than those using DA as the primary neurotransmitter. Previous studies have shown that Tg mice producing high levels of human cytosolic SOD (30), an enzyme that catalyzes the breakdown of superoxide ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) (31) are protected from the DA-depleting effects of MPTP (29), methamphetamine (32), and MDMA (33); therefore, SOD Tg mice were used in this study to determine the possible role of oxygen free radicals in neurotoxicity selectively associated with 5-HT and NE nerve terminals in nonstriatal brain regions.

Materials and Methods

Drugs and chemicals. 2'-NH_2-MPTP (free base) was synthesized at the National Institute of Mental Health (Bethesda, MD). The structure was verified by ^1H NMR and mass spectrometry. Purity was determined to be ~99% by gas chromatography. 2'-NH_2-MPTP was stored desiccated at 4° and checked periodically for stability by HPLC. [^3H]5-HT (36.9 Ci/mmol), [^{125}I]RTI-55 (2200 Ci/mmol) and [^{125}I]RTI-121 (2200 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA). GBR-12909 [1-[2-[*bis*(4-fluorophenyl)-methoxy]ethyl]-4-(3-phenylpropyl)piperazine], paroxetine, and LR1111 [1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)homopiperazine] were gifts from R. B. Rothman (National Institute on Drug Abuse, Baltimore, MD). Selegiline was obtained from Chinoin Pharmaceutical Works (Budapest, Hungary). All other chemicals were purchased from Sigma Chemical (St. Louis, MO) or a comparable source and were of analytical grade.

Animals. Non-Tg male CD-1 mice from Charles River Laboratories (Wilmington, MA) or SOD Tg male mice of the strain 218/3 (30) carrying multiple copies of the human Cu-Zn SOD gene, as described elsewhere (34), and weighing 30–45 g, were used for these experiments. SOD hetero-Tg mice showed a mean 3-fold increase in brain SOD activity in all brain regions previously examined, including the cortex, striatum, hippocampus, cerebellum, and spinal cord, whereas SOD homoTg mice showed a 5-fold increase in brain SOD activity relative to non-Tg mice (34).

Animals were housed in groups of three to five per cage with food and water *ad libitum* in a facility approved by the American Association for Accreditation of Laboratory Animal Care. Experimental protocols adhered to National Institutes of Health guidelines and were approved by the National Institute of Mental Health and the National Institute on Drug Abuse Animal Care and Use Committees.

Drug treatments. Because 2'-NH_2-MPTP had not been given to CD-1 mice in the past, pilot studies were conducted initially with 2'-NH_2-MPTP to determine an appropriate dose and route of administration for this strain of mouse, which is the background strain for the SOD Tg mice. In the first pilot study, 2'-NH_2-MPTP was administered subcutaneously in four injections of 20 mg/kg at 2-hr intervals; however, four of six mice receiving this dose regimen died within 1 hr of the last injection. In the second pilot study, 2'-NH_2-MPTP was administered intraperitoneally in four injections of 15 or 20 mg/kg at 2-hr intervals; only one mouse in the higher dose group died after intraperitoneal drug administration.

Based on the results of these pilot studies, 2'-NH_2-MPTP was administered to non-Tg mice, SOD-heteroTg mice, and SOD-homoTg mice at a dose of 4 × 15 mg/kg intraperitoneally at 2-hr intervals in a volume of 0.1 ml (eight mice per group). A second cohort of non-Tg, SOD-heteroTg, and SOD-homoTg mice received similarly timed injections of sterile saline (eight mice per group). Three weeks after treatment, mice were killed by cervical dislocation and their brains were removed rapidly and dissected on ice to obtain the following brain regions for neurochemical analysis: frontal cortex, hippocampus, striatum, brain stem, and hypothalamus. The remaining left hemisphere was frozen in isopentane on dry ice for autoradiography. Samples were stored at −70° pending analysis.

Neurochemical analysis. Brain region samples were analyzed for monoamine neurotransmitters and their metabolites by HPLC using electrochemical detection at +0.75 V with minor modifications (20). Briefly, individual samples were sonicated in 200–250 μl of 0.1 M perchloric acid and centrifuged at 7200 × *g* for 10 min; 50 μl of the resulting supernatant was injected onto a 10- × 4.6-mm Spherisorb 3-μm octadecyl sulfate reversed-phase chromatography column (Thomson Instruments, Springfield, VA) in a mobile phase containing 0.1 M citric acid, 8% acetonitrile, 0.5 g/l octanesulfonic acid, 0.3% triethylamine, and 10 μM EDTA at a flow rate of 0.7 ml/min. 5-HT, 5-HIAA, NE, DA, and the DA metabolites DOPAC and HVA were separated and detected in a single chromatogram and were quantitated as relative peak areas versus the internal standard, 5-hydroxy-*N*ω-methyltryptamine. Protein was determined by the method of Lowry *et al.* (35). Concentrations are expressed as ng/mg protein (mean ± standard error).

Autoradiographic determination of 5-HT and DA uptake sites. Cerebral hemispheres were sectioned coronally (20 μm) at −20° and thaw-mounted on gelatin-coated slides. For autoradiographic determination of 5-HT uptake sites, slides were incubated for 90 min at 4° with [^{125}I]RTI-55 (150,000 cpm/ml) in a 50 mM sodium phosphate buffer, pH 7.4. The radioligand was prepared in a phosphate-buffered protease cocktail, pH 7.4, containing 1 mg/ml bovine serum albumin, 25 μg/ml chymostatin, 25 μg/ml leupeptin, 100 μM EDTA, and 100 μM EGTA that was added to samples in a 1:10 dilution. LR1111 (1 μM) was used to inhibit binding of [^{125}I]RTI-55 to DA uptake sites (36). Nonspecific binding was determined in the presence of 1 μM paroxetine and represented <10% of the total binding. After incubation, slides were rinsed in fresh incubation buffer, dipped in ice-cold distilled water, and dried under a stream of

cool air. The slides were then apposed to radiosensitive films (Hyperfilm, Amersham, Arlington Heights, IL) along with plastic standards (^{125}I microscales; Amersham) for 5 days at 4°.

For determination of DA uptake sites, slides were incubated for 60 min at room temperature with [^{125}I]RTI-121 (100,000 cpm/ml) in an incubation buffer containing 137 mM NaCl, 2.7 mM KCl, 10.4 mM Na_2HPO_4, 1.76 mM KH_2PO_4, and 10 mM NaI. Nonspecific binding was determined in the presence of GBR-12909, and it represented <10% of the total binding. After incubation, slides were rinsed twice in fresh incubation buffer, dipped in ice-cold distilled water, and dried under a stream of cool air. The slides and plastic standards were apposed to radiosensitive films for 2 days at 4°. Both [^{125}I]RTI-121 and [^{125}I]RTI-55 binding were quantitated using standard curves generated from the ^{125}I microscales (NIH Image Software; National Institutes of Health) and are expressed as nCi/mg tissue (mean ± standard error).

5-HT uptake assay. Frontal cortex or hippocampus was dissected from the brains of non-Tg, SOD-heteroTg, and SOD-homoTg mice and was homogenized in 20 volumes of ice-cold 0.32 M sucrose with a motor-driven Teflon homogenizer and centrifuged at 800 × *g* for 10 min. The supernatant was transferred and recentrifuged at 20,000 × *g* for 10 min. The resulting pellet was resuspended gently in ice-cold 0.32 M sucrose to a final volume of 100 ml/g original wet weight tissue. Assay buffer (600 μl) containing 126 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 16 mM Na-phosphate, pH 7.4, 1.4 mM $MgSO_4$, 2 mg/ml dextrose, and 0.2 mg/ml ascorbic acid, tissue (100 μl), and 10 μM pargyline (100 μl) were preincubated for 10 min at 30°. [^{3}H]5-HT at a final concentration of 5 nM was added to each sample to begin the uptake, during which time samples were incubated for 3 min at 30°C. The reaction was terminated by rapid filtration through glass microfiber filters, size B, presoaked in 0.05% polyethylenimine. Nonspecific uptake was determined in the presence of 1 μM citalopram, which represented ~20% of the total uptake.

MAO-A enzyme assay. MAO-A activity was measured using tryptamine as the substrate by HPLC with electrochemical detection of the product indole-3-acetic acid at +0.8 V. Crude mitochondrial fractions were prepared from whole brains of non-Tg, SOD-heteroTg, and SOD-homoTg mice by homogenization in 0.32 M sucrose (10 ml/g wet weight) using a motor-driven Teflon pestle followed by centrifugation at 12,000 × *g* for 10 min. The resulting pellets were resuspended once in sucrose and centrifuged at 21,000 × *g* for 20 min. The tissue was then resuspended in 0.1 M phosphate buffer (20 ml/g wet weight; pH 7.4), centrifuged at 12,000 × *g* for 10 min, decanted, and stored at −70°.

On the day of analysis, brain tissue was rehomogenized briefly in assay buffer containing 0.1 M phosphate, pH 7.4, and 3 mM NAD (30 ml/g original wet weight). To irreversibly inhibit MAO-B, 600-μl aliquots of tissue were preincubated with 0.25 μM selegiline for 15 min at 37° (37). After preincubation, varying concentrations of tryptamine (4–50 μM) were added, along with alcohol dehydrogenase (0.13 units/ml) to convert the intermediate product, indole-3-acetaldehyde, to an electrochemically active final product, indole-3-acetic acid, in the presence of NAD. Samples were incubated at 37° for 30 min in a final volume of 1 ml, after which they were deprotonated with 250 μl of 0.5 M $HClO_4$ and centrifuged at 7200 × *g* for 10 min; 50 μl of each supernatant was injected onto a 10-cm × 4.6-mm Advantage 3-μm octadecyl sulfate reversed-phase chromatography column (Thomson Instruments) in a mobile phase containing 0.1 M monochloroacetic acid, 0.55 g/l octanesulfonic acid, 0.3% triethylamine, 25% acetonitrile, and 10 μM EDTA, pH 2.6, at a flow rate of 0.5 ml/min. Protein was measured as indicated in Neurochemical analysis.

Statistics. Data were analyzed initially by one-way analysis of variance using the Statistical Analysis System (SAS Institute, Carey, NC). Significant differences between 2'-NH_2-MPTP-treated groups and their respective control groups are indicated by *t* test probabilities corrected using the Bonferoni factor (*G*) where $G = n \cdot (n - 1)/2$, where *n* is the number of treatment groups. Differences among the three groups of 2'-NH_2-MPTP-treated mice were analyzed by the *post hoc* Scheffé test. All values are expressed as mean ± standard error; differences of $p < 0.05$ are considered statistically significant.

Results

Dose finding pilot study with 2'-NH_2-MPTP. Both 4 × 15 mg/kg intraperitoneal and 4 × 20 mg/kg intraperitoneal 2'-NH_2-MPTP depleted 5-HT and NE levels to 10–25% of control in frontal cortex and hippocampus in CD-1 mice (Fig. 2), without significantly affecting striatal DA (control, 92 ± 12; 4 × 15 mg/kg, 73 ± 2; 4 × 20 mg/kg, 116 ± 16 ng/mg protein). Because there was no consistent increase in the magnitude of the neurochemical deficit produced by the higher 4 × 20 mg/kg dose of 2'-NH_2-MPTP, the lower 4 × 15 mg/kg dose was chosen for use in the main study.

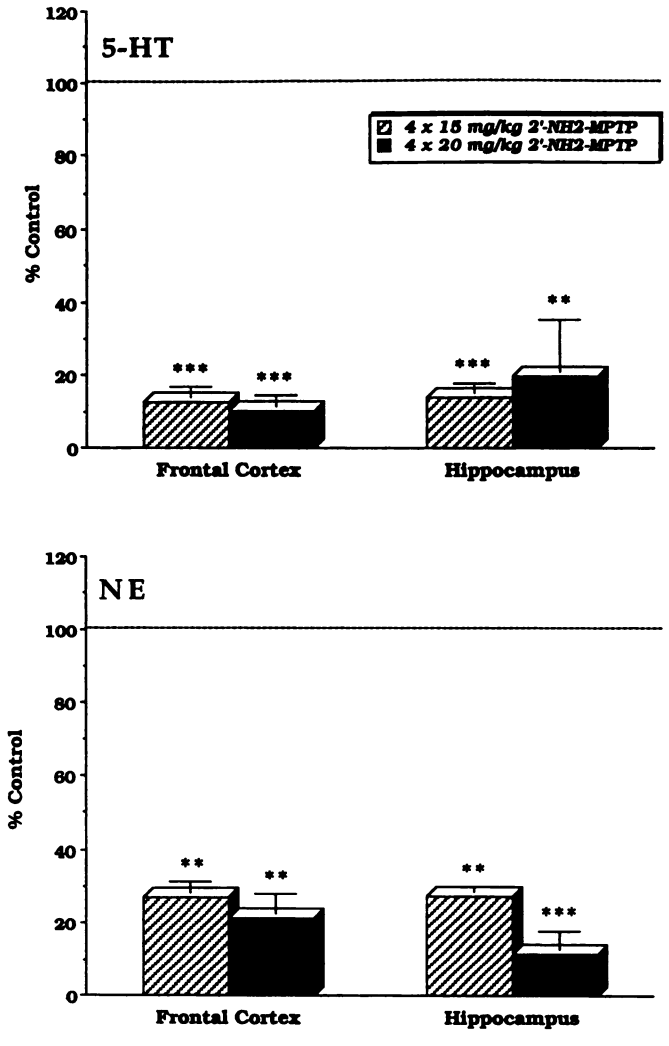

Fig. 2. Changes in 5-HT and NE in non-Tg CD-1 mice 3.5 weeks after 2'-NH_2-MPTP. Mice were injected four times with 15 mg/kg 2'-NH_2-MPTP (four mice) or 20 mg/kg 2'-NH_2-MPTP (four mice) at 2-hr intervals. Control animals (four mice) received four injections of saline; 3.5 weeks later, 5-HT and NE were measured by HPLC as described in Neurochemical analysis. Results shown are percentages of the following control group values in cortex and hippocampus, respectively (ng/mg protein, mean ± standard error): 5-HT, 9.2 ± 0.4 and 5.8 ± 0.6; NE, 4.0 ± 0.5 and 4.6 ± 0.4. Probabilities indicated in the figure are as follows: **, $p < 0.01$ different from control.; ***$p < 0.001$ different from control.

Base-line comparison between non-Tg, SOD-heteroTg, and SOD-homoTg mice. To examine the possible effects of increased SOD activity on base-line monoamine levels and 5-HT uptake site density, data obtained from the saline-treated non-Tg, SOD-heteroTg, and SOD-homoTg mice were compared. Analysis of variance revealed no significant differences between the three groups of mice, with the minor exceptions of cortical 5-HT, which was decreased in SOD-homoTg mice by 30% relative to the non-Tg group, hippocampal 5-HIAA, which was increased in SOD-heteroTg mice by 30% relative to non-Tg mice, and 5-HT uptake sites, which were decreased by 33% in temporal cortex layers 1–2 of the SOD-homoTg mice relative to the non-Tg group ($p < 0.05$ in all cases). No significant differences were found in the levels of NE, DA, DOPAC, or HVA among the three groups of mice. Although these data indicated that there were no consistent changes in the monoaminergic systems of the SOD-Tg mice used for this study, all of the data that follow on 2′-NH_2-MPTP-treated mice are expressed as a percentage of respective control group data and were analyzed statistically as such; i.e., the 5-HT levels in 2′-NH_2-MPTP-treated SOD-heteroTg mice are expressed as a percentage of the 5-HT levels in saline-treated SOD-heteroTg mice.

Effects of increased SOD activity on 2′-NH_2-MPTP-induced depletions in 5-HT and NE. As shown in Fig. 3, administration of 2′-NH_2-MPTP to CD-1 non-Tg mice markedly reduced 5-HT to ~30% of control in frontal cortex and hippocampus ($p < 0.001$) and to 75% of control in striatum ($p < 0.01$) 3 weeks after treatment. Cortical and hippocampal NE were also reduced significantly to ~20% of control ($p < 0.001$) in the 2′-NH_2-MPTP-treated non-Tg mice. The 5-HT metabolite, 5-HIAA, was depleted to 40% of control in frontal cortex and hippocampus ($p < 0.001$).

Fig. 3 also shows that the SOD Tg mice were protected from the neurotoxic effects of 2′-NH_2-MPTP in a gene dose-dependent manner. Analysis of variance revealed a significant group effect across the non-Tg, SOD-heteroTg, and SOD-homoTg groups for 5-HT levels [$F(5,42) = 17.82$, $p < 0.001$; $F(5,42) = 29.03$, $p < 0.001$], 5-HIAA levels [$F(5,42) = 31.57$, $p < 0.001$; $F(5,42) = 45.79$, $p < 0.001$], and for NE levels [$F(5,41) = 11.49$, $p < 0.001$; $F(5,42) = 16.90$, $p < 0.001$] in frontal cortex and hippocampus, respectively. 2′-NH_2-MPTP-treated SOD-heteroTg mice showed intermediate depletions in 5-HT to 50–60% of control, in NE to 30–40% of control and in 5-HIAA to 45–60% of control in frontal cortex and hippocampus ($p < 0.001$ versus saline-treated SOD-heteroTg mice, Fig. 3). The SOD-homoTg mice were nearly completely protected from the effects of 2′-NH_2-MPTP on 5-HT, 5-HIAA, and NE levels in the frontal cortex and hippocampus; the only statistically significant difference was seen in cortical NE, which was reduced to 70% of the saline-treated SOD-homoTg level ($p < 0.05$).

Post hoc analysis revealed that cortical and hippocampal 5-HT levels in the 2′-NH_2-MPTP-treated SOD-homoTg mice were significantly greater than those seen in the 2′-NH_2-MPTP-treated non-Tg mice ($p < 0.05$, Fig. 3) and that 5-HIAA and NE levels in these brain regions in the 2′-NH_2-MPTP-treated SOD-homoTg group were significantly greater than those seen in both the 2′-NH_2-MPTP-treated non-Tg group and the 2′-NH_2-MPTP-treated SOD-heteroTg group ($p < 0.05$; Fig. 3).

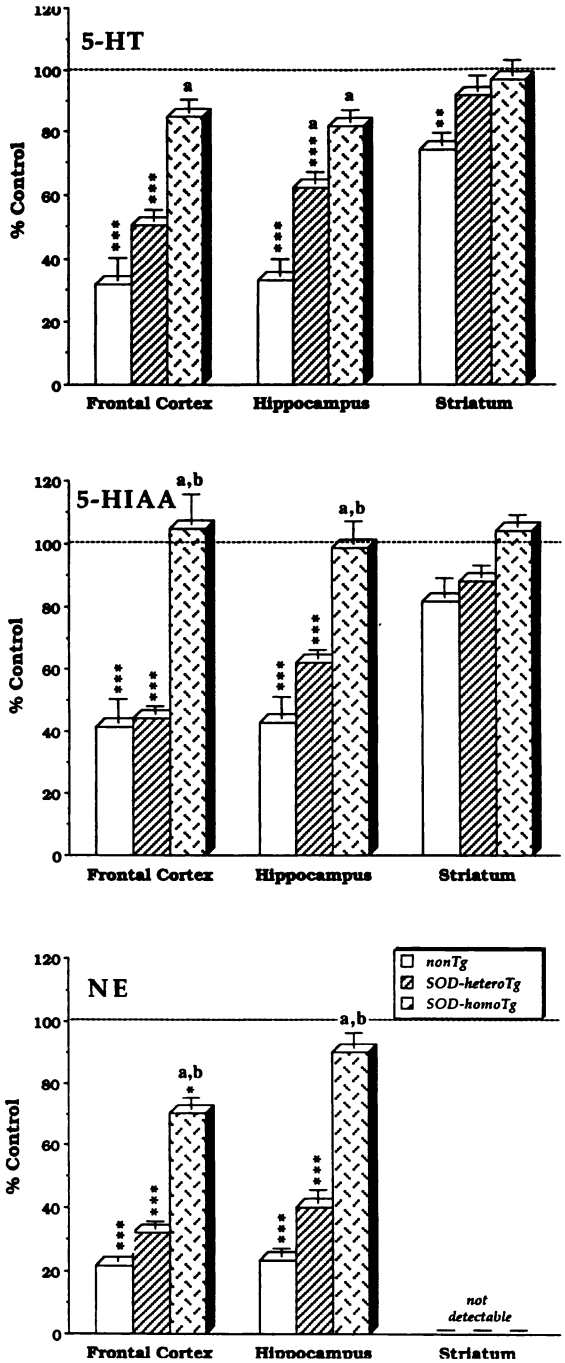

Fig. 3. Changes in 5-HT, 5-HIAA, and NE in SOD-Tg mice 3 weeks after 2′-NH_2-MPTP. Mice were injected four times with 15 mg/kg 2′-NH_2-MPTP (eight mice per group) at 2-hr intervals whereas control animals (eight mice per group) received four similarly timed injections of saline. Three weeks after treatment, 5-HT, 5-HIAA, and NE were measured by HPLC as described in Neurochemical analysis. Results shown are percentages of the following control group values in cortex, hippocampus, and striatum, respectively (ng/mg protein, mean ± standard error): non-Tg [5-HT, 14 ± 1, 8.0 ± 0.6, 4.9 ± 0.3; 5-HIAA, 2.2 ± 0.2, 4.0 ± 0.3, 3.1 ± 0.2; NE, 5.8 ± 0.4, 5.8 ± 0.5, and not detectable]; SOD-heteroTg [5-HT, 14 ± 1, 7.1 ± 0.2, 4.5 ± 0.2; 5-HIAA, 2.8 ± 0.5, 5.2 ± 0.1, 3.1 ± 0.3; NE, 6.1 ± 0.8, 5.8 ± 0.3, and not detectable]; SOD-homoTg [5-HT, 9.9 ± 0.5, 8.1 ± 0.4, 4.3 ± 0.1; 5-HIAA, 2.1 ± 0.08, 4.6 ± 0.3, 2.8 ± 0.1; NE, 5.8 ± 0.2, 6.4 ± 0.3, and not detectable]. Probabilities indicated in the figure are as follows: *, $p < 0.05$ different from respective control group; **, $p < 0.01$ different from respective control group; ***, $p < 0.001$ different from respective control group; *a*, $p < 0.05$ different from 2′-NH_2-MPTP-treated non-Tg group; *b*, $p < 0.05$ different from 2′-NH_2-MPTP-treated SOD-heteroTg group.

With regard to the effects of 2′-NH_2-MPTP in brain stem and hypothalamus (not pictured), the analysis of variance was only significant for 5-HT in the hypothalamus [$F(5,42) = 7.54$, $p < 0.001$], in which 5-HT levels in the 2′-NH_2-MPTP-treated animals were as follows: non-Tg, 63.4% ± 3.3% ($p < 0.001$); SOD-heteroTg, 73.2% ± 4.3% ($p < 0.05$); and SOD-homoTg, 89.3% ± 11% (not significant) versus respective saline-treated controls. *Post hoc* analysis revealed no significant differences in 5-HT, 5-HIAA, or NE levels among the 2′-NH_2-MPTP-treated groups in hypothalamus and brain stem.

Effects of 2′-NH_2-MPTP treatment on [^{125}I]RTI-55-labeled 5-HT uptake sites. Table 1 and Fig. 4 illustrate the effects of 2′-NH_2-MPTP administration on [^{125}I]RTI-55-labeled 5-HT uptake sites in cortical and hippocampal subregions and in the substantia nigra. Most subregions in the non-Tg mice showed marked 2′-NH_2-MPTP-induced reductions in 5-HT uptake site density to 21–35% of control ($p < 0.001$; Table 1), with the exception of parietal cortex layers 1–2, which were decreased to 56% of control ($p < 0.01$; Table 1), and substantia nigra, which was unchanged. SOD-heteroTg mice were only moderately affected by treatment with 2′-NH_2-MPTP, showing significant decreases in 5-HT uptake site binding density to 56–83% of control in most areas, except parietal cortex layers 1–2 and substantia nigra, which were unaffected, and dentate gyrus, which showed a decrease to 33% of control (Table 1). 5-HT uptake site density in the SOD-homoTg mice was relatively unaffected by 2′-NH_2-MPTP treatment; this group showed significant decreases only in temporal cortex layer 3 (70% of control, $p < 0.05$; Table 1) and layers 4–6 (64% of control, $p < 0.001$; Table 1).

Post hoc analysis of the 5-HT uptake site binding data showed that the density of these sites in the 2′-NH_2-MPTP-treated SOD-homoTg mice was significantly greater than that seen in the 2′-NH_2-MPTP-treated non-Tg mice (Table 1) with the exception of layers 1–2 and 3 of the parietal cortex. In addition, the 5-HT uptake site density was also significantly greater in many subregions of the SOD-heteroTg group than that observed in the non-Tg group after treatment with 2′-NH_2-MPTP (Table 1).

Effects of 2′-NH_2-MPTP treatment on DA levels and [^{125}I]RTI-121-labeled DA uptake sites. The data in Table 2 show that 2′-NH_2-MPTP was without significant effect on striatal DA and its metabolites DOPAC and HVA in the three groups of mice treated with 2′-NH_2-MPTP. In contrast to the effects of 2′-NH_2-MPTP on 5-HT uptake sites, Table 3 shows that 2′-NH_2-MPTP caused no changes in the density of [^{125}I]RTI-121-labeled DA uptake sites in striatum in either the non-Tg, SOD-heteroTg, or the SOD-homoTg mice.

MAO-A activity in SOD-Tg mice. Because 2′-NH_2-MPTP requires oxidation by MAO-A to produce neurotoxicity (21), the activity of MAO-A was assessed in non-Tg versus SOD-heteroTg and SOD-homoTg mice to determine whether decreased MAO-A activity might account for the diminished 2′-NH_2-MPTP toxicity observed in SOD-Tg mice. Fig. 5 shows that no statistically significant differences were seen in either the K_m or the V_{max} of MAO-A using tryptamine as a substrate in brain mitochondrial preparations from the three different types of mice.

[^{3}H]5-HT uptake in SOD-Tg mice. Selective uptake of 2′-NH_2-MPTP or its MAO-derived metabolite into serotonergic neurons via the 5-HT transporter is also a critical step in

TABLE 1

Effects of 2′-NH_2-MPTP on regional [^{125}I]RTI-55 binding to 5-HT uptake sites

Each value represents the mean ± standard error of five to seven mice and is expressed in nCi/mg of tissue. Numbers in parentheses are a percentage of each respective control group mean value.

	Non-Tg/Sal	Non-Tg/2′A	HeteroTg/Sal	HeteroTg/2′A	HomoTg/Sal	HomoTg/2′A
Parietal cortex 1–2	0.51 ± 0.05	0.28 ± 0.03 (56%)[b]	0.43 ± 0.05	0.48 ± 0.05 (112%)[d]	0.49 ± 0.03	0.41 ± 0.04 (83%)
Parietal cortex 3	2.61 ± 0.35	0.73 ± 0.10 (28%)[c]	2.35 ± 0.41	1.57 ± 0.23 (67%)	2.65 ± 0.17	1.62 ± 0.04 (61%)
Parietal cortex 4–6	3.06 ± 0.33	0.65 ± 0.11 (21%)[c]	2.27 ± 0.18	1.29 ± 0.21 (57%)[a]	2.94 ± 0.17	2.10 ± 0.41 (71%)[d]
Cingulate cortex	0.86 ± 0.91	0.29 ± 0.04 (34%)[c]	0.80 ± 0.07	0.56 ± 0.04 (70%)[d]	0.73 ± 0.04	0.56 ± 0.03 (77%)[d]
Temporal cortex 1–2	0.84 ± 0.08	0.21 ± 0.04 (25%)[c]	0.66 ± 0.08	0.37 ± 0.04 (56%)[a]	0.56 ± 0.04[a]	0.56 ± 0.05 (99%)[d,e]
Temporal cortex 3	1.20 ± 0.09	0.30 ± 0.04 (25%)[c]	1.04 ± 0.11	0.66 ± 0.07 (63%)[b,d]	1.06 ± 0.07	0.74 ± 0.02 (70%)[a,d]
Temporal cortex 4–6	1.22 ± 0.08	0.43 ± 0.04 (35%)[c]	1.19 ± 0.09	0.69 ± 0.04 (58%)[c,d]	1.24 ± 0.05	0.79 ± 0.03 (64%)[c,d]
Hippocampus CA1	2.12 ± 0.20	0.62 ± 0.05 (29%)[c]	1.82 ± 0.07	1.28 ± 0.07 (70%)[a,d]	2.00 ± 0.20	1.65 ± 0.16 (83%)[d]
Hippocampus CA2	2.93 ± 0.05	0.76 ± 0.09 (26%)[c]	2.40 ± 0.21	1.99 ± 0.11 (83%)[d]	2.64 ± 0.14	2.68 ± 0.27 (101%)[d]
Hippocampus CA3	4.70 ± 0.08	1.09 ± 0.18 (23%)[c]	4.85 ± 0.36	3.27 ± 0.20 (68%)[b,d]	5.39 ± 0.55	4.62 ± 0.32 (86%)[d]
Dentate gyrus	3.17 ± 0.22	0.85 ± 0.11 (27%)[c]	3.84 ± 0.44	1.25 ± 0.10 (33%)[c]	2.94 ± 0.17	2.22 ± 0.12 (76%)[d,e]
Substantia nigra	5.26 ± 0.18	5.10 ± 0.12 (97%)	6.26 ± 0.21	6.43 ± 0.23 (103%)	5.72 ± 0.57	5.92 ± 0.20 (103%)

[a] $p < 0.05$ versus respective control group.
[b] $p < 0.01$ versus respective control group.
[c] $p < 0.001$ versus respective control group.
[d] $p < 0.05$, versus 2′-NH_2-MPTP-treated non-Tg group.
[e] $p < 0.05$ versus 2′-NH_2-MPTP-treated SOD-heteroTg group.

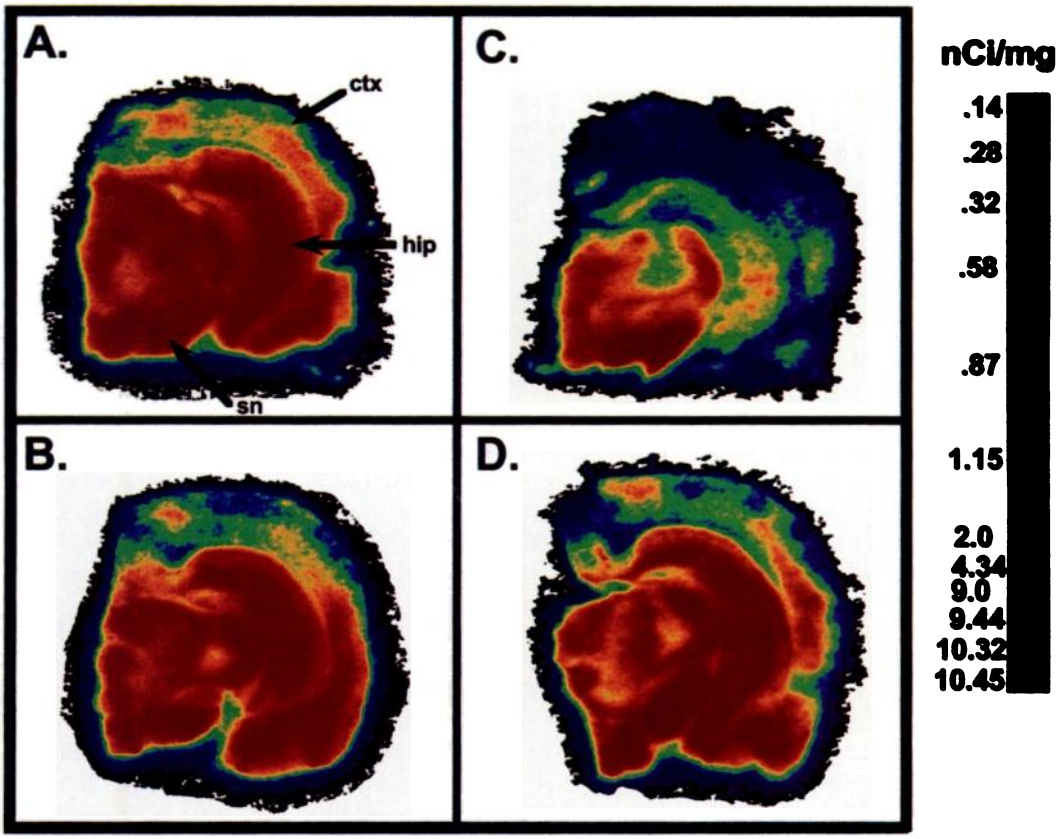

Fig. 4. Autoradiograms of [^{125}I]RTI-55 binding to 5-HT uptake sites 3 weeks after treatment. The changes in 5-HT uptake site densities in cortical (*ctx*) and hippocampal (*hip*) subregions at the level of the substantia nigra (*sn*) are representative of animals from the following groups: A, non-Tg/saline-treated. B, SOD-homoTg/saline-treated. C, Non-Tg/2′-NH_2-MPTP-treated. D, SOD-homoTg/2′-NH_2-MPTP-treated.

TABLE 2

Effects of 2′-NH_2-MPTP in striatum

Each value represents the mean ± standard error of eight mice and is expressed in ng/mg of protein. Numbers in parentheses are a percentage of each respective control group mean value.

	DA	DOPAC	HVA
Non-Tg/saline	181 ± 12	15.3 ± 2.0	12.3 ± 0.7
Non-Tg/2′-NH_2-MPTP	159 ± 6 (88%)	15.3 ± 1.2 (100%)	13.6 ± 0.9 (111%)
SOD-heteroTg/saline	158 ± 11	14.1 ± 2.1	13.6 ± 1.0
SOD-heteroTg/2′-NH_2-MPTP	153 ± 10 (97%)	16.7 ± 2.2 (118%)	12.4 ± 0.5 (91%)
SOD-homoTg/saline	163 ± 5	18.3 ± 1.9	12.2 ± 0.6
SOD-homoTg/2′-NH_2-MPTP	196 ± 15 (120%)	16.1 ± 0.7 (88%)	13.8 ± 0.7 (113%)

TABLE 3

Effects of 2′-NH_2-MPTP on regional [^{125}I]RTI-121 binding to dopamine uptake sites

Each value represents the mean ± standard error of five to seven mice and is expressed in nCi/mg of tissue. Numbers in parentheses are a percentage of each respective control group mean value.

	Non-Tg/Sal	Non-Tg/2′A	HeteroTg/Sal	HeteroTg/2′A	HomoTg/Sal	HomoTg/2′A
Dorsomedial striatum	3.3 ± 0.2	3.7 ± 0.2 (111%)	4.1 ± 0.2	3.9 ± 0.2 (96%)	3.7 ± 0.1	3.8 ± 0.1 (103%)
Dorsolateral striatum	2.8 ± 0.2	3.0 ± 0.2 (110%)	3.2 ± 0.1	3.2 ± 0.1 (99%)	2.9 ± 0.1	3.1 ± 0.1 (109%)
Ventromedial striatum	2.3 ± 0.1	2.2 ± 0.2 (94%)	2.8 ± 0.1	2.6 ± 0.1 (91%)	2.6 ± 0.1	2.9 ± 0.1 (109%)
Ventrolateral striatum	2.0 ± 0.1	2.0 ± 0.1 (104%)	2.2 ± 0.1	2.3 ± 0.1 (101%)	2.1 ± 0.1	2.4 ± 0.1 (116%)

the mechanism of action of 2′-NH_2-MPTP (21, 22). Active uptake of 5 nM [^{3}H]5-HT into synaptosomes prepared from non-Tg versus SOD-heteroTg and SOD-homoTg mice was compared and found to be not statistically different in frontal cortex and hippocampus, respectively: non-Tg, 46 ± 2 and 34 ± 1; SOD-heteroTg, 52 ± 1 and 33 ± 1; and SOD-homoTg, 45 ± 3 and 32 ± 2 (pmol/g original wet weight, six determinations).

Discussion

The main findings of the present report may be summarized as follows: (a) 2′-NH_2-MPTP reduces cortical and hippocampal 5-HT and NE as well as the density of cortical and hippocampal 5-HT uptake sites in the CD-1 mouse strain; (b) increased superoxide dismutase activity protects against

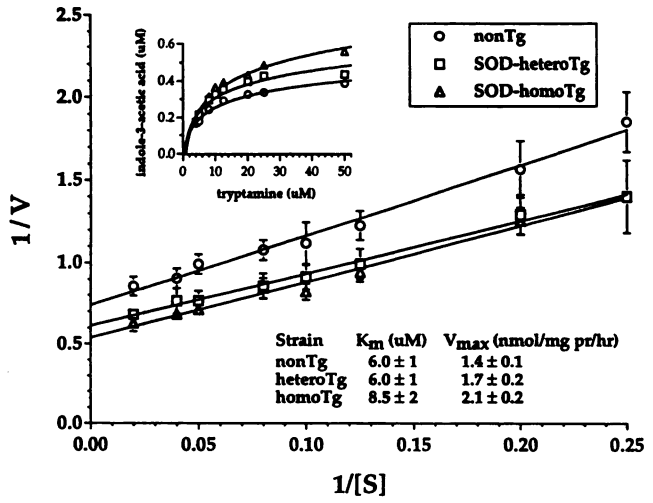

Fig. 5. Oxidation of tryptamine by MAO-A in non-Tg versus SOD-heteroTg and SOD-homoTg mice. Mitochondrial preparations from whole brains of the three types of mice were prepared as indicated in MAO-A enzyme assay and incubated with varying concentrations of tryptamine (4–50 μM). *Inset*, representative saturation curves from non-Tg, SOD-heteroTg, and SOD-homoTg mice run on the same day. *Lineweaver-Burk plot*, mean value for each substrate concentration run in duplicate on three separate occasions.

these toxic effects in a gene dose-dependent manner; and (c) neither DA levels nor the density of DA uptake sites are affected by the administration of 2'-NH_2-MPTP.

In Swiss-Webster and C57Bl/6 mice, 4 × 20 mg/kg 2'-NH_2-MPTP caused cortical and hippocampal 5-HT and NE decreases on the order of 60–90% (21, 22). In the CD-1 strain, 4 × 15 mg/kg 2'-NH_2-MPTP caused similar magnitude losses of 5-HT and NE (70–80%), which were generally not augmented by the higher (4 × 20 mg/kg) dose, except for the possibility of greater NE reductions observed with the higher intraperitoneal dose of 2'-NH_2-MPTP (Fig. 1). A previous study using 4 × 15 mg/kg 2'-NH_2-MPTP in Swiss-Webster mice has shown that this dose produced only minimal (25%) reductions in cortical and hippocampal 5-HT levels accompanied by 50–70% reductions in cortical and hippocampal NE (21). These results suggest that CD-1 mice may be more sensitive to the toxic effects of 2'-NH_2-MPTP on forebrain 5-HT and NE levels than Swiss-Webster mice and when combined with previous results on comparative data between Swiss-Webster and C57Bl/6 mice (21), the strains tested to date may be ordered as follows, with regard to their sensitivity to 2'-NH_2-MPTP: CD-1 > Swiss-Webster > C57Bl/6.

Previous studies with 2'-NH_2-MPTP have demonstrated long-lasting decreases in 5-HT and NE levels in frontal cortex and hippocampus persisting for at least 6 months after treatment, and these data were proposed to be indicative of long term neurotoxicity (21). The present study expands on these findings by demonstrating a decrease in [^{125}I]RTI-55-labeled 5-HT uptake sites after treatment with 2'-NH_2-MPTP. Loss of the 5-HT uptake site has been proposed as an indirect measure of 5-HT terminal degeneration and has been observed after the administration of other serotonergic neurotoxins in the rat (38).

The most striking finding of this study is that mice with increased SOD activity are protected from the neurotoxic effects of 2'-NH_2-MPTP on cortical and hippocampal 5-HT and NE nerve terminals. Previous investigations using SOD Tg mice have shown that these animals are protected from the dopaminergic neurotoxicity of MPTP, methamphetamine, and MDMA (29, 32, 33). In addition, protection against serotonergic neurotoxicity in striatum caused by methamphetamine was demonstrated recently using quantitative [^{125}I]RTI-55 binding to the 5-HT uptakes site (39), although a similar protection was not seen with respect to methamphetamine-induced decreases in striatal 5-HT levels (32). Because the latter study only made use of SOD-heteroTg mice, it remains to be determined whether the further increase in SOD activity seen in the SOD-homoTg mice would protect them from methamphetamine-induced striatal 5-HT depletions.

Most investigations on dopaminergic neurotoxins have been directed necessarily at effects seen in striatum due to the rich DA innervation of this brain region, and controversy continues as to whether oxyradicals are a major contributory factor leading to dopaminergic neurotoxicity, especially with regard to MPTP (40). However, excess DA release after systemic administration of MPTP, methamphetamine, and MDMA is well documented (41–44), and this observation led Chiueh *et al.* (26) to suggest that autoxidation of this excess DA might be a source of neurotoxic oxygen radicals.

The fact that SOD Tg mice are protected from the 5-HT- and NE-depleting effects of 2'-NH_2-MPTP suggests that, in addition to the nigrostriatal DA system, oxygen-based radicals may be important in neurotoxic mechanisms operating in serotonergic and noradrenergic pathways in brain regions such as frontal cortex and hippocampus. Unlike the data on DA, however, less evidence is available that is suggestive of physiologically relevant 5-HT or NE autoxidation as a possible source of oxyradicals (45–47). Another scenario could be envisioned in which 2'-NH_2-MPTP, or possibly the corresponding pyridinium, might inhibit the mitochondrial complex I enzyme NADH-ubiquinone oxidoreductase, as is the case for 1-methyl-4-phenylpyridinium and many of its analogs (48–50). This, in turn, could lead to the production of excess superoxide radicals (27, 51), and subsequent generation of other toxic oxygen species such as hydroxyl radicals and/or H_2O_2, all of which could be directly neurotoxic to 5-HT and NE terminals or could drive other oxidative reactions. For example, 2'-NH_2-MPTP administration followed by increased superoxide formation might lead to the oxidation of 5-HT to 5,6- and 5,7-dihydroxytryptamine, the former of which has been detected in rat brain after *p*-chloramphetamine and methamphetamine administration (52, 53) and both of which are known to cause serotonergic neurotoxicity. In any case, the observation that SOD-Tg mice are protected from 2'-NH_2-MPTP, as well as the other dopaminergic neurotoxins mentioned above, suggests that superoxide radicals probably are critical in the mechanisms of actions of these different types of neurotoxins.

Because 5-HT-selective uptake inhibitors or MAO-A inhibitors prevent 2'-NH_2-MPTP-induced decreases in 5-HT (21, 22), attenuation of the serotonergic neurotoxicity observed in 2'-NH_2-MPTP-treated mice with increased levels of SOD could theoretically be due to decreased 5-HT uptake or MAO-A activity in these mice. Schickler *et al.* (54) reported that SOD-Tg mice showed diminished 5-HT uptake in platelets; however, a more recent study demonstrated that in brain, both cortical 5-HT and NE uptake are actually increased by ~25% in SOD-Tg mice (55). These studies were

conducted in a strain of SOD-Tg mice bred from a different founder than the strain used in this study, so the results are not directly applicable. For the 218/3 strain of SOD-Tg mice used here, our data on base-line monoamine levels, 5-HT uptake site density, and [^{3}H]5-HT uptake itself, as well as base-line MAO-A activity, strongly support the conclusion that the monoaminergic systems in this strain of SOD-Tg mice are relatively unaltered with respect to wild-type CD-1 mice.

In summary, this is the first report of protection from noradrenergic neurotoxicity and from serotonergic neurotoxicity outside of striatum in Tg mice that overexpress normal human Cu-Zn SOD, emphasizing a possible role for superoxide radicals not only in nigrostriatal dopaminergic neurotoxicity as has been demonstrated previously but also for neurotoxicity in serotonergic and noradrenergic terminal fields in cortex and hippocampus.

Acknowledgments

We thank Dr. Boris Yagen for assistance in the synthesis of 2′-NH_2-MPTP, Amy M. Mach and Theresa A. Kopajtic for their expert technical assistance, and Dr. C. C. Chiueh for his helpful advice.

References

1. Davis, G. C., A. C. Williams, S. P. Markey, M. H. Ebert, E. D. Caine, C. M. Reichert, and I. J. Kopin. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res.* **1**:249–254 (1979).
2. Langston, J. W., P. Ballard, J. W. Tetrud, and I. Irwin. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science (Washington D. C.)* **219**:979–980 (1983).
3. Herkenham, M., M. D. Little, K. Bankiewicz, S. C. Yang, S. P. Markey, and J. N. Johannessen. Selective retention of MPP+ within the monoaminergic systems of the primate brain following MPTP administration: an in vivo autoradiographic study. *Neuroscience* **40**:133–158 (1991).
4. Burns, R. S., C. C. Chiueh, S. P. Markey, M. H. Ebert, D. M. Jacobowitz, and I. J. Kopin. A primate model of Parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* **80**:4546–4550 (1983).
5. Heikkila, R. E., A. Hess, and R. C. Duvoisin. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science (Washington D. C.)* **224**:1451–1453 (1984).
6. Sundstrom, E., and G. Jonsson. Pharmacological interference with the neurotoxic action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in the mouse. *Eur. J. Pharmacol* **110**: 293–299 (1985).
7. Booth, R. G., A. Trevor, T. P. Singer, and N. J. Castagnoli. Studies on semirigid tricyclic analogues of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J. Med. Chem.* **32**:473–477 (1989).
8. Bradbury, A. J., B. Costall, A. M. Domeney, B. Testa, P. G. Jenner, C. D. Marsden, and R. J. Naylor. The toxic actions of MPTP and its metabolite MPP+ are not mimicked by analogues of MPTP lacking an N-methyl moiety. *Neurosci. Lett.* **61**:121–126 (1985).
9. Finnegan, K. T., I. Irwin, L. E. Delanney, G. A. Ricaurte, and J. W. Langston. 1,2,3,6-tetrahydro-1-methyl-4-(methylpyrrol-2-yl)pyridine: studies on the mechanism of action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J. Pharmacol. Exp. Ther.* **242**:1144–1151 (1987).
10. Fuller, R. W., L. S. Hemrick, and D. W. Robertson. Comparison of 1-methyl-4-(p-chlorophenyl)-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and p-chloroamphetamine as monoamine depletors. *Res. Commun. Chem. Pathol. Pharmacol.* **50**:57–65 (1985).
11. Harik, S. I., N. J. Riachi, M. A. Hritz, M. S. Berridge, and L. M. Sayre. Development of fluorinated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs with potent nigrostriatal toxicity for potential use in positron emission tomography studies. *J. Pharmacol. Exp. Ther.* **266**:790–795 (1993).
12. Hemrick, L. S., D. W. Robertson, J. J. Krushinski, and R. W. Fuller. Persistent depletion of striatal dopamine and cortical norepinephrine in mice by 1-methyl-4-phenyl-1,2,3,6-tetrahydro-3-pyridinol (MPTP-3-OL), an analog of MPTP. *Life Sci.* **50**:573–582 (1992).
13. Naiman, N., H. Rollema, E. Johnson, and N. J. Castagnoli. Studies on 4-benzyl-1-methyl-1,2,3,6-tetrahydropyridine, a nonneurotoxic analogue of the Parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Chem. Res. Toxicol.* **3**:133–138 (1990).
14. Nilsson, K., A. Hallberg, E. Pileblad, and A. Tunek. Investigation of the possible dopaminergic toxicity of 1-methyl-3-phenyl-1,2,3,6-tetrahydropyridine, an isomer to the neurotoxin MPTP. *Pharmacol. Toxicol.* **69**:38–42 (1991).
15. Riachi, N. J., P. K. Arora, L. M. Sayre, and S. I. Harik. Potent neurotoxic fluorinated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs as potential probes in models of Parkinson disease. *J. Neurochem.* **50**:1319–1321 (1988).
16. Rollema, H., R. G. Booth, and N. J. Castagnoli. In vivo dopaminergic neurotoxicity of the 2-beta-methylcarbolinium ion, a potential endogenous MPP+ analog. *Eur. J. Pharmacol.* **153**:131–134 (1988).
17. Youngster, S. K., P. K. Sonsalla, B. A. Sieber, and R. E. Heikkila. Structure-activity study of the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. I. Evaluation of the biological activity of MPTP analogs. *J. Pharmacol. Exp. Ther.* **249**:820–828 (1989).
18. Johannessen, J. N., J. M. Savitt, C. J. Markey, J. P. Bacon, A. Weisz, D. S. Hanselman, and S. P. Markey. The development of amine substituted analogues of MPTP as unique tools for the study of MPTP toxicity and Parkinson's disease. *Life Sci.* **40**:697–704 (1987).
19. Heikkila, R. E., M. V. Kindt, P. K. Sonsalla, A. Giovanni, S. K. Youngster, K. A. McKeown, and T. P. Singer. Importance of monoamine oxidase A in the bioactivation of neurotoxic analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. USA* **85**:6172–6176 (1988).
20. Andrews, A. M., and D. L. Murphy. Sustained depletion of cortical and hippocampal serotonin and norepinephrine but not striatal dopamine by 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH_2-MPTP): a comparative study with 2′-CH3-MPTP and MPTP. *J. Neurochem.* **60**: 1167–1170 (1993).
21. Andrews, A. M., and D. L. Murphy. 2′-NH_2-MPTP in Swiss Webster mice: evidence for long term (six month) depletions in cortical and hippocampal 5-HT and NE, differential protection by selective uptake inhibitors or clorgyline, and functional changes in central 5-HT neurotransmission. *J. Pharmacol. Exp. Ther.* **267**:1432–1439 (1993).
22. Andrews, A. M., and D. L. Murphy. Fluoxetine and desipramine selectively attenuate 2′-NH_2-MPTP-induced depletions in serotonin and norepinephrine. *Eur. J. Pharmacol.* **250**:215–221 (1993).
23. Heikkila, R. E., L. Manzino, F. S. Cabbat, and R. C. Duvoisin. Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature (Lond.)* **311**: 467–469 (1984).
24. Markey, S. P., J. N. Johannessen, C. C. Chiueh, R. S. Burns, and M. A. Herkenham. Intraneuronal generation of a pyridinium metabolite may cause drug-induced Parkinsonism. *Nature (Lond.)* **311**:464–467 (1984).
25. Javitch, J. A., R. J. D'Amato, S. M. Strittmatter, and S. H. Snyder. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. Natl. Acad. Sci. USA* **82**:2173–2177 (1985).
26. Chiueh, C. C., G. Krishna, P. Tulsi, T. Obata, K. Lang, S. J. Huang, and D. L. Murphy. Intracranial microdialysis of salicylic acid to detect hydroxyl radical generation through dopamine autooxidation in the caudate nucleus: effects of MPP+. *Free Radical Biol. Med.* **13**:581–583 (1992).
27. Cleeter, M. W., J. M. Cooper, and A. H. Schapira. Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement. *J. Neurochem.* **58**:786–789 (1992).
28. Ali, S. F., S. N. David, G. D. Newport, J. L. Cadet, and W. Slikker, Jr. MPTP-induced oxidative stress and neurotoxicity are age-dependent: evidence from measures of reactive oxygen species and striatal dopamine levels. *Synapse (N. Y.)* **18**:27–34 (1994).
29. Przedborski, S., V. Kostic, L. V. Jackson, A. B. Naini, S. Simonetti, S. Fahn, E. Carlson, C. J. Epstein, and J. L. Cadet. Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. *J. Neurosci.* **12**:1658–1667 (1992).
30. Epstein, C. J., K. B. Avraham, M. Lovett, S. Smith, S. O. Elroy, G. Rotman, C. Bry, and Y. Groner. Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of dosage effects in Down syndrome. *Proc. Natl. Acad. Sci. USA* **84**:8044–8048 (1987).
31. Halliwell, B. Reactive oxygen species and the central nervous system. *J. Neurochem.* **59**:1609–1623 (1992).
32. Cadet, J. L., P. Sheng, S. Ali, R. Rothman, E. Carlson, and C. Epstein. Attenuation of methamphetamine-induced neurotoxicity in copper/zinc superoxide dismutase transgenic mice. *J. Neurochem.* **62**:380–383 (1994).
33. Cadet, J. L., B. Ladenheim, H. Hirata, R. B. Rothman, S. A. Ali, E. Carlson, C. Epstein, and T. H. Moran. Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse (N. Y.)* **21**:169–176 (1995).
34. Chan, P. H., C. J. Epstein, H. Kinouchi, H. Kamii, S. Imaizumi, G. Yang, S. F. Chen, J. Gafni, and E. Carlson. SOD-1 transgenic mice as a model for studies of neuroprotection in stroke and brain trauma. *Ann. N. Y. Acad. Sci. (Washington D. C.)* **738**:93–103 (1994).
35. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein

measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
36. Silverthorn, M. L., C. M. Dersch, M. H. Baumann, J. L. Cadet, J. S. Partilla, K. C. Rice, F. I. Carroll, K. M. Becketts, A. Brockington, and R. B. Rothman. Studies of the biogenic amine transporters. V. Demonstration of two binding sites for the cocaine analog [125 I]RTI-55 associated with the 5-HT transporter in rat brain membranes. *J. Pharmacol. Exp. Ther.* **273**: 213–222 (1995).
37. Sonsalla, P. K., S. K. Youngster, M. V. Kindt, and R. E. Heikkila. Characteristics of 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine-induced neurotoxicity in the mouse. *J. Pharmacol. Exp. Ther.* **242**:850–857 (1987).
38. Battaglia, G. Criteria for assessing drug-induced biochemical alterations and degeneration of serotonergic neurons, in *Serotonin: From Cell Biology to Pharmacology and Therapeutics* (R. Paoletti, P. M. Vanhoutte, N. Brunello, and F. M. Maggi, eds.). Kluwer Academic, Dordrecht, The Netherlands, 651–660 (1990).
39. Hirata, H., B. Ladenheim, R. B. Rothman, C. Epstein, and J. L. Cadet. Methamphetamine-induced serotonin neurotoxicity is mediated by superoxide radicals. *Brain Res.* **677**:345–347 (1995).
40. Bates, T. E., S. J. R. Heales, S. E. C. Davies, P. Boakye, and J. B. Clark. Effects of 1-methyl-4-phenylpyridinium on isolated rat brain mitochondria: evidence for a primary involvement of energy depletion. *J. Neurochem.* **63**:640–648 (1994).
41. O'Dell, S. J., F. B. Weihmuller, and J. F. Marshall. Methamphetamine-induced dopamine overflow and injury to striatal dopamine terminals: attenuation by dopamine D1 or D2 antagonists. *J. Neurochem.* **60**:1792–1799 (1993).
42. Baldwin, H. A., M. I. Colado, T. K. Murray, R. J. De Souza, and A. R. Green. Striatal dopamine release in vivo following neurotoxic doses of methamphetamine and effect of the neuroprotective drugs, chlormethiazole and dizocilpine. *Br. J. Pharmacol.* **108**:590–596 (1993).
43. Obata, T., Y. Yamanaka, and C. C. Chiueh. In vivo release of dopamine by perfusion of 1-methyl-4-phenylpyridinium ion in the striatum with a microdialysis technique. *Jpn. J. Pharmacol.* **60**:311–313 (1992).
44. Schmidt, C. J., C. K. Sullivan, and G. M. Fadayel. Blockade of striatal 5-hydroxytryptamine2 receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.* **62**:1382–1389 (1994).
45. Wrona, M. Z., D. Lemordant, L. Lin, C. L. Blank, and G. Dryhurst. Oxidation of 5-hydroxytryptamine and 5,7-dihydroxytryptamine. A new oxidation pathway and formation of a novel neurotoxin. *J. Med. Chem.* **29**:499–505 (1986).
46. Graham, D. G. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol. Pharmacol.* **14**:633–643 (1978).
47. Graham, D. G., S. M. Tiffany, W. R. Bell, Jr., and W. F. Gutknecht. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Mol. Pharmacol.* **14**:644–653 (1978).
48. Arora, P. K., N. J. Riachi, G. C. Fiedler, M. P. Singh, F. Abdallah, S. I. Harik, and L. M. Sayre. Structure-neurotoxicity trends of analogues of 1-methyl-4-phenylpyridinium (MPP+), the cytotoxic metabolite of the dopaminergic neurotoxin MPTP. *Life Sci.* **46**:379–390 (1990).
49. Saporito, M. S., R. E. Heikkila, S. K. Youngster, W. J. Nicklas, and H. M. Geller. Dopaminergic neurotoxicity of 1-methyl-4-phenylpyridinium analogs in cultured neurons: relationship to the dopamine uptake system and inhibition of mitochondrial respiration. *J. Pharmacol. Exp. Ther.* **260**: 1400–1409 (1992).
50. Nicklas, W. J., I. Vyas, and R. E. Heikkila. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci.* **36**:2503–2508 (1985).
51. Hasegawa, E., K. Takeshige, T. Oishi, Y. Murai, and S. Minakami. 1-Methyl-4-phenylpyridinium (MPP+) induces NADH-dependent superoxide formation and enhances NADH-dependent lipid peroxidation in bovine heart submitochondrial particles. *Biochem. Biophys. Res. Commun.* **170**:1049–1055 (1990).
52. Axt, K. J., D. L. Commins, G. Vosmer, and L. S. Seiden. *alpha*-Methyl-p-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation. *Brain Res.* **515**:269–276 (1990).
53. Commins, D. L., K. J. Axt, G. Vosmer, and L. S. Seiden. Endogenously produced 5,6-dihydroxytryptamine may mediate the neurotoxic effects of para-chloroamphetamine. *Brain Res.* **419**:253–261 (1987).
54. Schickler, M., H. Knobler, K. B. Avraham, O. Elroy-Stein, and Y. Groner. Diminished serotonin uptake in platelets of transgenic mice with increased Cu/Zn-superoxide dismutase activity. *Embo. J.* **8**:1385–1392 (1989).
55. Schwartz, P. J., U. V. Berger, and J. T. Coyle. Mice transgenic for copper/zinc superoxide dismutase exhibit increased markers of biogenic amine function. *J. Neurochem.* **65**:660–669 (1995).

Send reprint requests to: Dr. Anne M. Andrews, Laboratory of Clinical Science, NIMH 10/3D-41, 10 Center Drive MSC 1264, Bethesda, MD 20892-1264. E-mail: ama@helix.nih.gov