2'-NH₂-MPTP in Swiss Webster Mice: Evidence for Long-Term (6-Month) Depletions in Cortical and Hippocampal Serotonin and Norepinephrine, Differential Protection by Selective Uptake Inhibitors or Clorgyline and Functional Changes in Central Serotonin Neurotransmission

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ABSTRACT

The i.p. administration of 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH₂-MPTP; 4 × 20 mg/kg) to Swiss Webster mice caused substantial decreases in cortical and hippocampal 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid and norepinephrine (NE) measured 1 week post-treatment. Compared with the authors' previously reported results in C57BL/6 mice, these effects were significantly greater in hippocampus (80-90% vs. 60%) and of a similar magnitude in frontal cortex (60-75%). A long-term study showed that cortical and hippocampal 5-HT, 5-hydroxyindoleacetic acid and NE were still decreased 40% to 50% 6 months after treatment. Regional brain dopamine was essentially unchanged during the 6-month period. Pretreatment with the 5-HT-selective uptake inhibitors, fluoxetine or paroxetine, or with the NE-selective uptake inhibitor, desipramine, prevented decreases in cortical and hippocampal 5-HT and NE, respectively, 3 weeks after 2'-NH₂-MPTP (4 × 20 mg/kg). In addition, pretreatment with the monoamine oxidase type-A inhibitor, clorgyline, also prevented the more modest decreases in 5-HT and NE caused by 4 × 15 mg/kg 2'-NH₂-MPTP. Selegiline, a monoamine oxidase-B inhibitor, did not provide similar protection. Lastly, 2'-NH₂-MPTP administered 3 weeks earlier, abolished hypothermia caused by the serotonin agonist, m-chlorophenylpiperazine, which provided preliminary evidence for an associated functional change in the central serotonergic system. Together, these data suggest that 2'-NH₂-MPTP is a novel agent capable of producing long-lasting depletions in forebrain 5-HT and NE but not dopamine in two different strains of mice by some mechanisms that resemble those of the parent dopamine-depleting neurotoxin, MPTP.

MPTP is now known to cause long-lasting dopaminergic neurotoxicity in humans (Davis et al., 1979; Langston et al., 1985), nonhuman primates (Burns et al., 1983; Herkenham et al., 1991) and mice (Heikkila et al., 1984a; Sundström et al., 1987). We recently reported that a novel MPTP analog, 2'-NH₂-MPTP, produced depletions sustained for 3 weeks in cortical and hippocampal 5-HT and NE without affecting the levels of striatal DA in C57BL/6 mice (Andrews and Murphy, 1993). Among other compounds known to deplete 5-HT or NE, some, such as p-chlorophenylalanine (Dewar et al., 1992; Koe and Weissman, 1966) and reserpine (Sanders-Bush, 1977), deplete monoamines through biochemical mechanisms without causing neuronal damage. Others, such as 5,7-dihydroxytryptamine, PCA, 6-hydroxydopamine (6-OHDA) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) (Jönsson, 1980) are believed to produce sustained depletions in 5-HT or NE as the result of long-lasting neurotoxic processes.

The present study was designed to provide both neurochemical and functional evidence to help evaluate the hypothesis that the substantial decrements in cortical and hippocampal 5-HT and NE after 2'-NH₂-MPTP treatment may reflect long-term neurotoxicity. In this regard, the effects of 2'-NH₂-MPTP on monoamine levels were evaluated over an extended 6-month period. An assessment of possible serotonergic functional changes was determined by evaluation of the hypothermia

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ABBREVIATIONS: 2'-NH₂-MPTP, 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPPT, 1-methyl-4-phenylpyridinium; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NE, norepinephrine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; HPLC, high-performance liquid chromatography; HPLC-ECD, HPLC/electrochemical detection; m-CPP, m-chlorophenylpiperazine; PCA, p-chloroamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MAO, monoamine oxidase.
caused by the 5-HT agonist m-CPP after 2'-NH₂-MPTP treatment.

In addition, this study investigated whether 2'-NH₂-MPTP is similar to MPTP in two important aspects of its mechanism of action. It has been shown that, to cause dopamine depletion, MPTP must first be oxidized by MAO-B and that the toxicity of MPTP can be prevented by inhibition of MAO-B by pargyline or selegiline (formerly known as deprenyl; Heikkila et al., 1984b; Markey et al., 1984). In addition, the resulting metabolite of MPTP, MPP⁺, is selectively taken up by the DA transporter; therefore, MPTP-induced toxicity also can be attenuated by catecholamine uptake inhibitors such as mazindol, nomifensine or amfoteric acid (Javitch et al., 1985; Mayer et al., 1986; Melamed et al., 1985; Sundström and Jonsson, 1985). By way of comparison with MPTP, we studied the effects of the selective MAO inhibitors, selegiline and clorgyline, on the depletions in 5-HT and NE caused by 2'-NH₂-MPTP. Clorgyline, a highly selective inhibitor of MAO-A, was studied and other 2'-substituted MPTP analogs were found to be substrates for MAO-A and -B (Heikkila et al., 1988; Youngster et al., 1989). We also hypothesized that the serotonergic and noradrenergic transporters might be involved in the toxicity of 2'-NH₂-MPTP; therefore, we evaluated the effects of the 5-HT-selective uptake inhibitors, fluoxetine and paroxetine, and the NE-selective uptake inhibitor, desipramine, on 2'-NH₂-MPTP-induced toxicity.

Methods

Materials. 2'-NH₂-MPTP was synthesized by Ecochem Research (Chaska, MN). The identity of 2'-NH₂-MPTP was verified by gas chromatography-mass spectroscopy and [H]-nuclear magnetic resonance. It was stored desiccated at 70°C and checked periodically for stability by HPLC. The following drugs were generous gifts: selegiline, Somerset Pharmaceuticals (Denville, NJ); clorgyline, May and Baker (Essex, England); fluoxetine, Lilly Lilly (Indianapolis, IN); and paroxetine, Smith Kline Beecham (Harlow, Essex, England). Desipramine and m-CPP were purchased from Aldrich Chemical (Milwaukee, WI) and all other drugs and chemicals were obtained from Sigma (St. Louis, MO).

Animal treatments. Swiss Webster male mice from Taconic Farms (Germantown, NY) weighing 25 to 35 g at the start of the study were housed under standard laboratory conditions with food and water ad libitum. In all studies, 2'-NH₂-MPTP was dissolved in sterile saline and administered in four injections at 2-hr intervals. Control animals received four similarly timed injections of saline. This dosing regimen was chosen so that direct comparisons could be made with existing literature on MPTP and another 2'-substituted analog, 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'-CH₃-MPTP), which we previously compared with 2'-NH₂-MPTP (Andrews and Murphy, 1993). In dose-response experiments, we observed >80% mortality rates after a single injection of 40 or 60 mg/kg i.p. of 2'-NH₂-MPTP in C57BL/6 mice; however, a cumulative dose of 40 mg/kg (given over 6 hr at 10 mg/kg × 4) led only to modest 25% to 30% reductions in cortical 5-HT, 5-HIAA and NE (Andrews and Murphy, in press, 1993). These observations led us to choose an 80-mg/kg cumulative dose (20 mg/kg × 4, calculated as the free base) for most experiments in this study. All injections were administered i.p. in a volume of 0.1 ml. For the long-term neurochemical study, four cohorts of mice were treated with four injections of 20 mg/kg of 2'-NH₂-MPTP and each cohort consisting of 2'-NH₂-MPTP-treated mice and saline-treated control mice was sacrificed at 1, 3, 9 and 24 weeks post-treatment.

Four injections of 15 mg/kg of 2'-NH₂-MPTP were administered after pretreatment with the MAO inhibitors selegiline or clorgyline (2.5 mg/kg i.p.) given the day before 2'-NH₂-MPTP. Preliminary experiments in C57BL/6 mice indicated that death resulted when 20-mg/kg injections of 2'-NH₂-MPTP were administered after pretreatment with MAO inhibitors (>70% mortality rate after the first injection of 2'-NH₂-MPTP, n=9; Andrews and Murphy, 1993). To avoid this potential problem in Swiss Webster mice, which were at least as sensitive to 2'-NH₂-MPTP as were C57BL/6 mice, 2'-NH₂-MPTP was administered at a lower 15-mg/kg dose in this experiment.

In a separate experiment, fluoxetine, paroxetine and desipramine were each administered in a single dose of 10 mg/kg i.p. (calculated as the salt) 90 min before the first of four injections of 20 mg/kg of 2'-NH₂-MPTP. The animals in these two latter experiments were sacrificed 3 weeks post-treatment.

In the 5-HT agonist challenge, m-CPP (10 mg/kg) was given to mice that had received either 2'-NH₂-MPTP (4 × 20 mg/kg) or saline 3 weeks earlier. The mice were then placed into the testing room temperature of 25.6 ± 0.3°C for 1 hr before the experiment. The colonic temperature was measured with a rectal probe and digital thermometer (Sensorex, Clifton, NJ). The thermocouple was inserted 2 cm into the colon of mice restrained gently by the tail. The temperature was measured two times before m-CPP administration to establish a base line and once every 30 min thereafter for a total of 3 hr. Published data suggest that this dose of m-CPP produces a statistically significant hypothermia in mice (Maj et al., 1988).

For studies that involved neurochemical analysis, the mice were killed by cervical dislocation and their brains were rapidly removed and dissected on ice. Brain regions rich in serotonergic and noradrenergic nerve terminals, namely, frontal cortex, hippocampus and hypothalamus and the brain stem, which contains the 5-HT and NE cell bodies, were obtained. Striatum, which contains a high proportion of dopaminergic innervation was also reserved for analysis. The brain regions were dissected using the Atlas of the Mouse Brain and Spinal Cord as a guide (Sidman et al., 1971) and were stored at −70°C pending analysis.

HPLC analysis. Brain region samples were analyzed for monoamine neurotransmitters and metabolites by HPLC-ECD at +0.85V by established methods (Mefford, 1981). Briefly, individual samples were sonicated in 200 to 250 μl of 0.1 M HClO₄ and centrifuged at 7200 g (12,000 rpm) for 10 min. Then 50 μl of each supernatant was injected onto a 10-cm × 4.6-mm Axxiom (Thomson Instruments, Springfield, VA) 3-μm octadeckysilicate reversed-phase chromatography column in a mobile phase containing 0.25 M citric acid, 5% to 9% acetonitrile, 450 mg/l of octanesulfonic acid, 0.3% to 0.4% triethylamine and 100 mg/l of EDTA. 5-Hydroxy-N-methyltryptamine was used as the internal standard and the calculations were performed by comparing the relative peak areas of sample peaks to external standards. 5-HT, 5-HIAA, NE, DA and the DA metabolites, DOPAC and HVA were all able to be measured in a single chromatogram. The order of elution and representative retention times were as follows: NE, 4.4; DOPAC, 6.5; DA, 7.7; 5-HIAA, 10.4; HVA, 12.7; 5-HT, 17.9; and 5-hydroxy-N-methyltryptamine, 21.2 min. Protein was measured by the method of Lowry et al. (Lowry et al., 1951). Concentrations are expressed in nanograms per milligram of protein (mean ± S.E.M.).

Data analysis. For neurochemical data, one-way analysis of variance was used to detect overall statistically significant differences among groups. For temperature data, a repeated-measures design was used. Individual t tests compared the least-squares means between groups. A P < .05 was considered significant after multiplication by a Bonferroni correction factor (q) where q = n · (n − 1)/2; n is the number of treatment groups.

Results

Long-term effects of a single regimen of 2'-NH₂-MPTP. 2'-NH₂-MPTP (4 × 20 mg/kg) or saline was administered to four cohorts of Swiss Webster mice that were later sacrificed at various time points to determine the long-term effects on brain monoamines. In frontal cortex and hippocampus, statistically significant decreases in 5-HT (75–85%, P <
The long-term effects of 2'-NH$_2$-MPTP on 5-HT, 5-HIAA and NE also were determined in other brain regions (fig. 2). In brain stem, significant decreases in 5-HT, 5-HIAA and NE on the order of 30% to 50% were measured at 1 and 3 weeks after 2'-NH$_2$-MPTP (P < .05 in all cases but one). These levels were only decreased 20% to 30% at 9 weeks (5-HT, not significant; 5-HIAA, P < .01; and NE, P < .05). At 24 weeks, there were no statistically significant changes in brain stem levels of 5-HT, 5-HIAA and NE compared with those in age-matched controls. The same general trend toward recovery occurred in hypothalamic 5-HT, 5-HIAA and NE levels with 2'-HIAA becoming significantly increased at 24 weeks (P < .05, fig. 2). In striatum, 5-HT was only significantly decreased at 9 weeks (35%, P < .05) and 5-HIAA at 1 and 3 weeks (25-30%, P < .01 and .05, respectively). Both were 100% of control at 24 weeks (fig. 2).

The effects of 2'-NH$_2$-MPTP on dopaminergic neurochemistry were negligible when studied over 6 months. Striatal levels of DA and its metabolites, DOPAC and HVA, were unchanged by the administration of 2'-NH$_2$-MPTP at all time points (fig. 3). Similarly, the levels of DA, DOPAC and HVA in the other brain regions analyzed were minimally affected over the 6-month period studied (data not shown).

Effects of pretreatment with MAO inhibitors on the changes in 5-HT and NE 3 weeks after 2'-NH$_2$-MPTP. When administered in four 15-mg/kg doses, 2'-NH$_2$-MPTP caused a 20% decrease in cortical 5-HT (P < .01), a 70% decrease in cortical NE (P < .001) and a 50% decrease in hippocampal NE (P < .001) as shown in figure 4. This lower dose of 2'-NH$_2$-MPTP was without effect on hippocampal 5-HT in Swiss Webster mice. The MAO-A inhibitor, clorgyline, significantly attenuated these 2'-NH$_2$-MPTP-induced decreases in NE (P < .001 vs. 2'-NH$_2$-MPTP) and cortical 5-HT (P < .05 vs. 2'-NH$_2$-MPTP). The MAO-B inhibitor selegiline did not show a similar protective effect as evidenced by the fact that levels of cortical 5-HT and cortical and hippocampal NE in mice pretreated with 2.5 mg/kg of selegiline were significantly decreased compared with those in control animals (P < .001) and were not significantly different from those in 2'-NH$_2$-MPTP-treated mice (fig. 4).

Effects of pretreatment with uptake inhibitors on the changes in 5-HT and NE 3 weeks after 2'-NH$_2$-MPTP. Pretreatment with both fluoxetine and paroxetine significantly attenuated the decreases in cortical and hippocampal 5-HT caused by four 20-mg/kg doses of 2'-NH$_2$-MPTP (P < .001 vs. 2'-NH$_2$-MPTP alone, fig. 5). Pretreatment with desipramine had a minimal effect on 5-HT levels, the changes in which were statistically significant in hippocampus only (P < .05 vs. 2'-NH$_2$-MPTP alone). This profile was reversed in the case of NE; desipramine pretreatment abolished NE decrements completely (P < .001 vs. 2'-NH$_2$-MPTP alone, fig. 5). Fluoxetine and paroxetine did not cause any significant changes in NE levels compared with those in the 2'-NH$_2$-MPTP-treated group.

5-HT agonist challenge in 2'-NH$_2$-MPTP-treated mice. The serotonin agonist m-CPP was administered to mice 3 weeks after treatment with 4 x 20 mg/kg of 2'-NH$_2$-MPTP or four injections of saline. m-CPP caused a 2°C decrease in body temperature in the saline-pretreated mice that was statistically significant when measured at 30, 60, 90 and 120 min after m-CPP (P < .001 at 30, 60 and 90 min; P < .05 at 120 min, fig. 6). This m-CPP-induced hypothermia was not ob-
served in the 2'-NH₂-MPTP-treated mice the body temperatures of which were not statistically different from animals who received saline in place of m-CPP (saline/saline group). At 30 and 60 min after m-CPP challenge, the mean colonic temperature in the 2'-NH₂-MPTP/m-CPP-treated group was significantly different from that of the saline/m-CPP-treated group (P<.001 at 30 min; P<.01 at 60 min).

**Discussion**

Previously, we reported that 2'-NH₂-MPTP caused a selective depletion of cortical and hippocampal 5-HT and NE in C57BL/6 mice in contrast to the parent compound, MPTP, and another 2'-substituted analog, 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (Andrews and Murphy, 1993). In this study, we extended these findings to include Swiss Webster mice. In comparative strain studies on MPTP, the C57BL/6 mouse was more sensitive to MPTP-induced neurotoxicity than was the Swiss Webster strain. DA was depleted to a greater extent (Giovanni et al., 1991; Sundstrom and Jonsson, 1985) and the 70% loss of cell bodies in the substantia nigra observed histochemically in C57BL/6 mice was not seen in the Swiss Webster strain (Sundström et al., 1987). By contrast, Swiss Webster mice were at least as sensitive to the depleting effects of 2'-NH₂-MPTP on 5-HT and NE as were C57BL/6 mice. In hippocampus, Swiss Webster mice showed significantly greater reductions in 5-HT, 5-HIAA and NE than did C57BL/6 mice after 2'-NH₂-MPTP (P<.01, C57BL/6 mouse data from Andrews and Murphy, 1993). In prior reports, regional analysis of brain monoamines indicated that MPTP produced a more selective lesion in C57BL/6 mice. Whereas cortical NE levels were decreased 50% in Swiss Webster mice after MPTP, C57BL/6 mice showed near-normal levels of NE in frontal cortex (Sundström et al., 1987). In the case of 2'-NH₂-MPTP, Swiss Webster and C57BL/6 mice showed a similar profile of monoamine depletions limited to the serotonergic and noradrenergic neurotransmitter systems with negligible effects on striatal DA and its metabolites (Andrews and Murphy, 1993).

In this study, we investigated the ability of 2'-NH₂-MPTP to affect monoamine levels over long periods. The most striking result from this experiment was the fact that, not only were cortical and hippocampal 5-HT and 5-HIAA still significantly decreased by 50% 2 months after 2'-NH₂-MPTP administration, but these levels remained unchanged for the subsequent 4 months. The trend was similar for NE, except that NE levels rose 20% over the final 4-month period. This relative plateau in the recovery from the effects of 2'-NH₂-MPTP suggests that 5-HT, 5-HIAA and NE reductions may be sustained for even longer periods. By contrast, data on MPTP showed that the levels of striatal DA in C57BL/6 mice completely recovered between 2 and 8 months post-treatment (Chiu et al., 1986).

6, 6); and 24 weeks (n = 6, 6), where n is the number of control animals and the number of 2'-NH₂-MPTP-treated animals, respectively. 5-HT, 5-HIAA and NE levels were measured by HPLC-ECD. The results shown are the percentages of the respective control group means ± S.E.M. The values for brain stem, hypothalamus and striatum, respectively, at 1 week were 5-HT, 4.96 ± 0.51, 8.97 ± 0.29 and 2.72 ± 0.58; 5-HIAA, 3.12 ± 0.38, 3.45 ± 0.17 and 2.50 ± 0.17; and NE, 4.64 ± 0.34, 25.66 ± 1.2 (and not detectable) ng/mg of protein. They are representative of control group means ± S.E.M. for the remaining time points. The probabilities were identified as *P < .05, **P < .01 and ***P < .001 for differences from the values in age-matched control groups.
Fig. 3. Long-term effects of 2′-NH₂-MPTP on DA, DOPAC and HVA in striatum of Swiss Webster Mice. Four cohorts of mice were injected i.p. four times with either 20 mg/kg of 2′-NH₂-MPTP or saline at 2-hr intervals. The animals were sacrificed at 1, 3, 9 and 24 weeks (see fig. 1 legend for the number of animals in each group). DA, DOPAC and HVA levels were measured by HPLC-ECD. The results shown are the percentages of respective control group means ± S.E.M. with representative values at 1 week being DA, 60.1 ± 4.5; DOPAC, 16.49 ± 1.9; and HVA, 11.5 ± 0.76 ng/mg protein. No statistical differences between groups at any time point were noted.

The duration of the depletions caused by a single day’s treatment with 2′-NH₂-MPTP seems to suggest that permanent damage to noradrenergic and serotonergic terminal fields in hippocampus and frontal cortex has occurred. However, confirmation of neurotoxicity in serotonergic and noradrenergic neuronal pathways will depend on corroborative evidence of other types, such as decreases in synthetic enzymes or uptake capacity, and morphological evidence, such as the presence of Fink-Heimer staining, decreases in the density of radiolabeled uptake sites and swollen immunoreactive fibers (Battaglia, 1990).

Nonetheless, it is unlikely that any pharmacological action of 2′-NH₂-MPTP is still present 6 months postadministration; therefore, these results provide evidence that 2′-NH₂-MPTP may be acting through a neurotoxic mechanism rather than a biochemical mechanism, such as the inhibition of synthetic enzymes. Based on comparative neurochemical and uptake site binding data obtained after treatment with MDMA, it was proposed that decreases in 5-HT concentrations may actually underestimate the magnitude of neurodegeneration (Battaglia et al., 1987); thus, the long-term damage to serotonergic and noradrenergic terminals in frontal cortex and hippocampus after 2′-NH₂-MPTP treatment may exceed 50%.

The results of this long-term study also showed that 2′-NH₂-MPTP had a differential effect on 5-HT and NE levels in various brain regions. In brain stem, striatum and hypothalamus, in which the initial effects of 2′-NH₂-MPTP were much less pronounced, 5-HT, 5-HIAA and NE concentrations returned to control levels 6 months after treatment. Regional analysis, therefore, suggested that the terminal fields found in frontal cortex and hippocampus were the most greatly and lastingly affected. This pattern was similar to that seen with the substituted amphetamines PCA, MDMA and fenfluramine in that 5-HT terminal fields appeared to be more affected than cell bodies (Dewar et al., 1992; Molliver and Molliver, 1990; O’Hearn et al., 1988). Further morphological studies, which are currently in progress, will provide evidence to assess whether the long-term neurochemical changes induced by 2′-NH₂-MPTP are the result of terminal degeneration.

Although 2′-NH₂-MPTP may be similar to the substituted amphetamines in the respect mentioned previously, it differs from this class of serotonergic neurotoxins in its ability to deplete NE as well as 5-HT. It also differs from PCA and MDMA, in particular, by virtue of the fact that these compounds are relatively nontoxic in mice as a result of an alternative metabolic deamination pathway for amphetamines in
this species (Peroutka, 1988; Steranka and Sanders-Bush, 1978; Stone et al., 1987). 2′-NH₂-MPTP may be useful as a systematically administered compound capable of producing long-term depletions in 5-HT and NE in mice, with selective attenuation of either the 5-HT or NE effects by pretreatment with various selective uptake inhibitors.

It has been suggested that, although long-term neurochemical deficits are essential pieces of evidence for assessing the neurotoxic potential of a putative chemical toxin, it is equally important to demonstrate that functional changes have occurred as a result of the suspected neurodegeneration (Battaglia, 1990). Maj et al. (1988) showed that the 5-HT agonist mCPP caused a dose-dependent hypothermia in mice that could be blocked by 5-HT₁B receptor antagonists. In our study, mice treated with 2′-NH₂-MPTP 3 weeks earlier did not exhibit the mCPP-induced hypothermia that occurred in animals that had received saline. Because thermoregulation is thought to be mediated by serotonergic neurons projecting to the preoptic area of the hypothalamus in rodents, damage to serotonergic terminals containing the 5-HT₁B presynaptic autoreceptors in this area of the brain could explain the lack of a functional response to the mCPP challenge. An alternative explanation involves the fact that mCPP may induce the release of 5-HT, in addition to its action as a direct agonist (Pettibone and Williams, 1984). As our neurochemical results showed, 5-HT was significantly reduced by 40% at 3 weeks post-treatment in hypothalamus; therefore, 2′-NH₂-MPTP-treated mice may show an attenuated response to mCPP as a result of decreased availability of 5-HT for release. Because 2′-NH₂-MPTP caused the greatest changes in cortical and hippocampal 5-HT, experiments that would identify serotonergic functional deficits mediated by these brain regions would be informative, as would experiments demonstrating similar deficits in transmission in noradrenergic neuronal pathways.

Finally, investigation of the similarities between 2′-NH₂-MPTP and the parent molecule, MPTP, demonstrated the importance of the serotonergic and noradrenergic transporters in the mechanism of action of 2′-NH₂-MPTP. Selective uptake by a particular neurotransmitter transporter was proved to be important for many known neurotoxins. The DA transporter
plays a critical role in the toxic selectivity of MPTP for dopaminergic neurons (Javitch et al., 1985; Mayer et al., 1986; Melamed et al., 1985; Sundstrom and Jonsson, 1985). Likewise, the toxic effects of PCA and MDMA on 5-HT nerve terminals can be prevented by pretreatment with 5-HT-selective reuptake inhibitors, such as fluoxetine and citalopram (Battaglia et al., 1988; Fuller et al., 1975) and the depletion of NE by 6-OHDA or DSP-4 can be attenuated by desipramine administration (Jonsson, 1980). In our study, a single dose of 10 mg/kg of desipramine was able to prevent completely the decreases in NE that occurred 3 weeks after 2′-NH₂-MPTP administration. In a similar fashion, fluoxetine and paroxetine pretreatment protected against 2′-NH₂-MPTP-induced depletions in 5-HT. Desipramine pretreatment also had a slightly significant effect on 5-HT levels in hippocampus of 2′-NH₂-MPTP-treated mice. This was an unexpected result because desipramine is considered a relatively weak inhibitor of the 5-HT transporter in vitro (Richelson and Pfening, 1984). In studies of the serotonin neurotoxin p-chloromethylamphetamine in vivo in rats, partial protection (~30%) of 5-HT reductions was observed after 25 mg/kg of desipramine (Meek et al., 1971), although no effect was found in a similar study of PCA with 10 mg/kg of desipramine (Fuller et al., 1975). We also showed that fluoxetine and desipramine are able to attenuate selectively the effects of 2′-NH₂-MPTP in C57BL/6 mice at 1 week post-treatment (Andrews and Murphy, in press, 1993). The differential neuroprotective effects of fluoxetine and paroxetine versus desipramine are most likely the result of the inhibition of active transport of 2′-NH₂-MPTP and/or an active metabolite, such as 2′-NH₂-MPP⁺ (by analogy to MPTP and MPP⁺) into serotonergic or noradrenergic nerve terminals by their respective reuptake systems.

A MAO-derived metabolite seems to be involved in the mechanism of action of 2′-NH₂-MPTP as evidenced by the prevention of 2′-NH₂-MPTP-induced decreases in 5-HT and NE after pretreatment with clorgyline. The role of MAO in 2′-NH₂-MPTP-induced toxicity parallels that seen for MPTP except for the fact that 2′-NH₂-MPTP may be a substrate for MAO-A by contrast with MPTP, which is almost exclusively a substrate for MAO-B (Heikkila et al., 1985). Therefore, although 2′-NH₂-MPTP is similar to MPTP in a general sense with regard to the initial steps of its mechanism of action, the requirements of 2′-NH₂-MPTP for MAO-A versus MAO-B and for the 5-HT and NE transporters versus the DA transporter make 2′-NH₂-MPTP different from MPTP in the specifics of its mechanism.

In conclusion, 2′-NH₂-MPTP has a long duration of action in 5-HT and NE terminal fields, with little or no recovery occurring in 5-HT, 5-HIAA and NE levels between 9 and 24 weeks. This evidence suggests that 2′-NH₂-MPTP is likely to cause long-term toxicity in serotonergic and noradrenergic nerve terminals, possibly associated with neurodegeneration of these structures. Morphological evidence will help to evaluate this hypothesis further. In addition, the specificity of 2′-NH₂-MPTP for serotonergic and noradrenergic terminals versus dopaminergic terminals can probably be explained, in part, by the ability of this compound, or more likely, an active metabolite produced by MAO-A to be actively transported by the 5-HT and NE reuptake systems.

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