

2'-NH₂-MPTP [1-Methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine] Depletes Serotonin and Norepinephrine in Rats: A Comparison with 2'-CH₃-MPTP [1-Methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine]

ERICA L. UNGER, PASCALE MAZZOLA-POMIETTO, DENNIS L. MURPHY, and ANNE M. ANDREWS

Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania (E.L.U., A.M.A.); Institut National de la Santé et de la Recherche Médicale, Marseilles, France (P.M.-P.); and Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland (D.L.M.)

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ABSTRACT

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analog, 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH₂-MPTP), depletes brain serotonin and norepinephrine in mice without affecting striatal dopamine. The present study was conducted to determine whether 2'-NH₂-MPTP would be similarly neurotoxic to rats. Four injections of 20 mg/kg 2'-NH₂-MPTP caused 80 to 90% depletions in serotonin and norepinephrine in frontal cortex and hippocampus in rats 1 week post-treatment. A lower dose of 2'-NH₂-MPTP (4 × 15 mg/kg) also produced large decrements in serotonin and norepinephrine levels and in serotonin transporter density measured 3 weeks after neurotoxin administration. Furthermore, this lower dose of 2'-NH₂-MPTP altered functional serotonin neurotransmission as evidenced by a 2-fold potentiation of 1-(3-chlorophenyl)-piperazine-2HCl-induced hyperthermia, an in-

dex of serotonergic denervation supersensitivity. At both doses, 2'-NH₂-MPTP was without effect on striatal dopamine. For comparison, additional rats were treated with a second 2'-substituted analog of MPTP, 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'-CH₃-MPTP), at 2 × 20 mg/kg. This dosing regimen causes substantial striatal dopamine depletion in mice. 2'-CH₃-MPTP had no effect on brain levels of serotonin, norepinephrine, or dopamine in rats. Together, these results demonstrate that rats are sensitive to the toxic effects of 2'-NH₂-MPTP but not to 2'-CH₃-MPTP at doses known to cause neurotoxicity in mice. Moreover, this study clearly shows that 2'-NH₂-MPTP can be utilized in rats as a tool to study the serotonergic and noradrenergic neurotransmitter systems.

Neurodegeneration in monoamine neurotransmitter systems is known to occur in disorders such as Parkinson's and

Alzheimer's diseases, and as part of the normal aging process (Gottfries, 1990; Palmer and DeKosky, 1993; Melamed et al., 1996; Miyawaki et al., 1997; Hornykiewicz, 1998; Meltzer et al., 1998; Perl et al., 1998; Parvizi et al., 2001). In this regard, selective neurotoxins are useful tools for studying neurodegenerative mechanisms. Studies on 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH₂-MPTP) have shown that this unique analog of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is capable of selectively destroying serotonin [5-hydroxytryptamine (5-HT)] and norepinephrine (NE) nerve terminals in mice while having no effect on striatal dopamine (DA) (Andrews and Murphy, 1993c). Depletions in 5-HT and NE in mice are most pronounced in frontal cortex and hippocampus and are selectively attenuated by 5-HT or NE uptake inhibitors, respectively (Andrews and Murphy, 1993a,b). 2'-NH₂-MPTP-induced neurotoxicity is also dependent on metab-

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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; 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; DA, dopamine; MAO, monoamine oxidase; MPP⁺, 1-methyl-4-phenylpyridinium; VMAT, vesicular monoamine transporter; *m*-CPP, 1-(3-chlorophenyl)-piperazine-2HCl; [¹²⁵I]RTI-55, [¹²⁵I]3β-(4-trimethylstannylphenyl)-tropane-2β-carboxylic acid methyl ester; GBR12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)homopiperazine; HPLC, high-performance liquid chromatography; 2'-CH₃-MPTP, 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.

olism by monoamine oxidase (MAO) type A and the production of superoxide radicals, and its effects persist for 6 months post-treatment (Andrews and Murphy, 1993a; Andrews et al., 1996).

The toxicity profile of 2'-NH₂-MPTP is very different from that of its parent compound MPTP, which is known to be toxic to dopamine-containing neurons in many animal species including humans (Langston et al., 1983), nonhuman primates (Burns et al., 1983), and mice (Heikkila et al., 1984). Even dogs (Johannessen et al., 1989), cats (Schneider et al., 1986), and goldfish (Poli et al., 1990) are susceptible to MPTP-induced dopamine depletion. Notably, however, rats are resistant to the effects of parenterally administered MPTP (Chiueh et al., 1984), and measurable dopaminergic neurotoxicity is observed only when MPP⁺, the toxic metabolite of MPTP, is infused directly into striatum at high concentrations (Giovanni et al., 1994b).

Previous studies have shown that MPP⁺ is taken up into adrenal chromaffin granules or neuronal synaptic vesicles by the vesicular monoamine transporter (VMAT1 or VMAT2, respectively) (Reinhard et al., 1987; Speciale et al., 1998). It has been hypothesized that vesicular sequestration is a protective mechanism and that enhanced vesicular MPP⁺ uptake in rats may account for their lack of sensitivity to MPTP-induced dopamine neurotoxicity (Russo et al., 1994). Recently, Staal et al. (2000) demonstrated that rats have a higher density of vesicular VMAT2 resulting in increased vesicular uptake of MPP⁺. Furthermore, inhibition of VMAT2 *in vivo* has been shown to increase the toxicity of intrastrially infused MPP⁺ in rats to some extent (Staal and Sonsalla, 2000).

In light of the overall insensitivity of rats to MPTP, the present investigation sought to determine whether this species would be similarly resistant to the serotonergic and noradrenergic neurotoxic effects of 2'-NH₂-MPTP. Rats were treated with one of two dosing regimens of 2'-NH₂-MPTP, both of which effectively deplete serotonin and norepinephrine in mice (Andrews and Murphy, 1993c; Andrews et al., 1996). The effects on monoamine neurochemistry in various brain regions were subsequently evaluated at two different time points. Serotonin transporter density was measured at the lower dose as an index of serotonergic terminal density (Battaglia, 1990; Andrews et al., 1996). Additionally, rats were challenged with the serotonin agonist 1-(3-chlorophenyl)-piperazine · 2HCl (*m*-CPP) to evaluate agonist-induced hyperthermia as a measure of denervation supersensitivity (Wozniak et al., 1989).

For comparison, the effects of a second *ortho*-substituted MPTP analog, 2'-CH₃-MPTP (1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine) were similarly studied. 2'-CH₃-MPTP has been shown to potently and selectively deplete striatal DA in mice (Youngster et al., 1986; Andrews and Murphy, 1993c). In addition, 2'-CH₃-MPP⁺ appears to be a substrate for VMAT2, albeit with lower affinity, which may account for the enhanced potency of 2'-CH₃-MPTP compared with MPTP (Reinhard and Daniels, 1992).

Materials and Methods

Materials. 2'-NH₂-MPTP was synthesized at the National Institute of Mental Health (Bethesda, MD). Paroxetine was a gift from Smith Kline & French (Philadelphia, PA). [¹²⁵I]RTI-55 was purchased from PerkinElmer Life Sciences (Boston, MA). 2'-CH₃-MPTP,

m-CPP, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)homopiperazine (GBR12935), and all other drugs and chemicals were purchased from Sigma/RBI (Natick, MA) and were of analytical grade.

Drug Treatments. Sprague-Dawley (CrI: CD(SD) BR) male rats from Charles River Laboratories Inc. (Wilmington, MA), weighing ~200 g at the beginning of the study, were housed 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 Animal Care and Use Committee. Initial doses of 2'-NH₂-MPTP and 2'-CH₃-MPTP were chosen based on their reported ability to produce long-term neurotoxicity in mice (Kindt et al., 1988; Andrews and Murphy, 1993a; Andrews et al., 1996). 2'-NH₂-MPTP was administered at 4 × 20 or 4 × 15 mg/kg doses at 2-h intervals. 2'-CH₃-MPTP was administered twice at 20 at a 6-h interval or 4 × 20 mg/kg at 2-h intervals. Control animals received similarly timed injections of sterile saline. Drugs were administered via the intraperitoneal route in a volume of 0.3 ml, and doses are reported as weight of the free base.

Neurochemistry. At the end of each experiment, rats were killed by decapitation, and their brains were rapidly removed and dissected over ice to obtain samples of frontal cortex, hippocampus, striatum, brain stem, and hypothalamus, which were stored at -70°C before analysis. Samples were analyzed for monoamine neurotransmitters and their metabolites by HPLC using electrochemical detection at +0.75 V as previously reported (Andrews and Murphy, 1993a). 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), NE, DA, and the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in a single chromatogram and were quantitated as relative peak areas versus 5-hydroxy-*N*ω-methyltryptamine as the internal standard. Protein was determined by the method of Lowry et al. (1951).

Autoradiographic Determination of 5-HT Uptake Sites. In rats treated with 4 × 20 mg/kg 2'-NH₂-MPTP, the cortex and lateral striatum remaining after dissection for neurochemistry was sectioned sagittally at 20 μm (-20°C) and thaw-mounted on gelatin-coated slides. Slides were incubated for 90 min at 4°C with [¹²⁵I]RTI-55 ([¹²⁵I]3β-(4-trimethylstannylphenyl)-tropane-2β-carboxylic acid methyl ester) (2200 Ci/mmol; PerkinElmer Life Sciences) at 150,000 cpm/ml in a sodium phosphate buffer, pH 7.4. The radioligand was prepared in a protease inhibitor cocktail 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 (Silverthorn et al., 1995). GBR12935 (Sigma/RBI) was used at 1 μM to inhibit binding of [¹²⁵I]RTI-55 to DA uptake sites. Nonspecific binding was determined in the presence of 1 μM paroxetine (Smith Kline & French) and represented <10% of the total binding. Following incubation, slides were rinsed (twice for 5 min) in fresh, cold phosphate buffer (50 mM, pH 7.4), desalted in ice-cold distilled water, and dried under a stream of cool air (fan). The slides were then apposed to radiossensitive films (Hyperfilm βmax; Amersham Biosciences Inc., Piscataway, NJ) along with plastic standards (¹²⁵I-labeled microscales; Amersham Biosciences) for 48 h at 4°C. Films were developed, and the images were digitized and analyzed using NIH Image software (National Institutes of Health, Bethesda, MD).

Hyperthermic Effect of *m*-CPP. Body temperature was recorded with a digital thermometer equipped with a rectal temperature probe which was inserted approximately 2.5 cm into the colon (Sensortek, Clifton, NJ). Animals were placed in the testing room at least 1 h before baseline temperature measurement (ambient temperature 22.5 ± 1°C) and were habituated to the probe during several exposures the day before the experiment. *m*-CPP (1.4 mg/kg) was injected *i.p.* in a volume of 0.1 ml/kg 11 days post-treatment with 2'-NH₂-MPTP (Wozniak et al., 1989).

Statistics. Data (means ± S.E.M.) were analyzed by one-way analysis of variance with drug as the independent variable using the Statistical Analysis System (SAS Institute, Carey, NC). Probabilities

<0.05 were considered significant findings. One-tailed tests were used for the temperature data since prior studies documented that serotonergic lesions produced by 5,7-dihydroxytryptamine markedly enhanced *m*-CPP-induced core temperature increases in rats (Murphy et al., 1991)

Results

Acute Behavioral Reactions. Within approximately 1 min of each injection, 4 × 20 mg/kg 2'-NH₂-MPTP caused piloerection, salivation, Straub tail, flattened body posture, intermittent walking, forepaw treading, wet-dog shakes, and noise sensitivity. In one rat, tonic-clonic seizure was noted after the third dose of 2'-NH₂-MPTP, and after the fourth dose in the remaining animals (20 min after injection). Rats administered 4 × 15 mg/kg 2'-NH₂-MPTP exhibited similar, although less severe, behavioral symptoms with no indication of seizure. Rats injected with 2 × 20 mg/kg 2'-CH₃-MPTP showed acute symptoms that were readily distinguishable from the 2'-NH₂-MPTP- and saline-treated groups. They were stationary for the most part and remained separated from each other. Some of these animals pointed their noses upward or occasionally crept around the cage floor.

Effects of 2'-NH₂-MPTP or 2-CH₃-MPTP on Monoamine Neurotransmitter Levels. In the first experiment, rats were killed 1 week after 4 × 20 mg/kg 2'-NH₂-MPTP, 2 × 20 mg/kg 2'-CH₃-MPTP, or saline. Analysis of variance revealed significant treatment effects in frontal cortex and hippocampus, respectively, for 5-HT [$F(2,6) = 64.8, p < 0.0001$; $F(2,5) = 22.2, p < 0.001$], 5-HIAA [$F(2,6) = 54.8, p < 0.0001$; $F(2,6) = 13.7, p < 0.01$], and NE [$F(2,6) = 11.5, p < 0.001$; $F(2,6) = 16.9, p < 0.001$]. In 2'-NH₂-MPTP-treated rats, 5-HT concentrations were depleted to ~10% of control, 5-HIAA concentrations were reduced to 15 to 25% of control, and NE was depleted to ~15% of control in frontal cortex and hippocampus. In brain stem, 5-HT was decreased to 60% of control [$F(2,6) = 12.3, p < 0.01$]. 2'-NH₂-MPTP had no effect on DA or its metabolites DOPAC and HVA in striatum (Table 1). By contrast, rats given 2 × 20 mg/kg 2'-CH₃-MPTP showed no statistically significant effects on 5-HT, 5-HIAA, or NE concentrations (Fig. 1) or on striatal DA or its metabolites (Table 1).

A second experiment was conducted to determine whether a larger cumulative dose of 2'-CH₃-MPTP administered on a schedule similar to that of 2'-NH₂-MPTP would result in depletions in monoamine neurotransmitter levels. 4 × 20 mg/kg 2'-CH₃-MPTP proved lethal to rats, and none survived longer than 2 h following the last injection.

A final experiment was conducted to determine whether a lower dose of 2'-NH₂-MPTP (4 × 15 mg/kg) would have measurable effects on 5-HT and NE levels. Analysis of variance for frontal cortex and hippocampus, respectively, re-

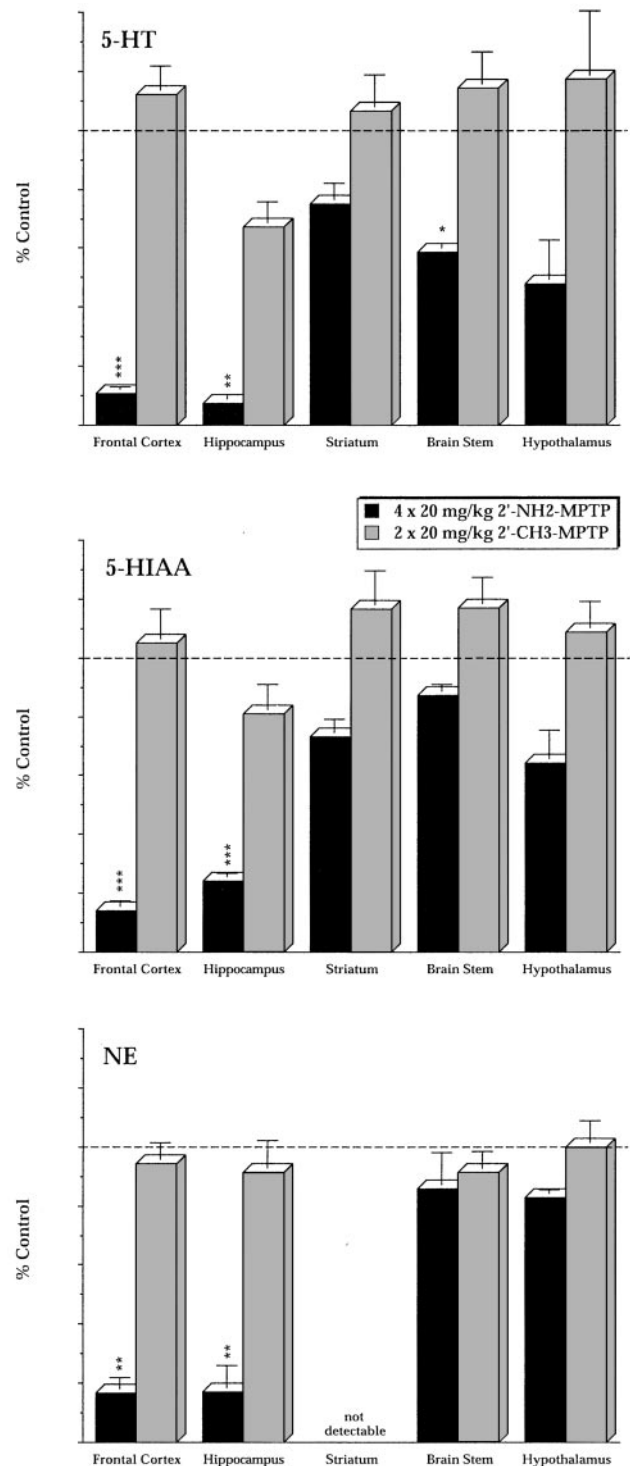


Fig. 1. Changes in 5-HT, 5-HIAA, and NE 1 week after four doses (4 × 20 mg/kg) of 2'-NH₂-MPTP or two doses (2 × 20 mg/kg) of 2'-CH₃-MPTP. Rats were injected with 4 × 20 mg/kg 2'-NH₂-MPTP ($n = 4$) at 2-h intervals or with 2 × 20 mg/kg 2'-CH₃-MPTP ($n = 3$) at a 6-h interval. Control animals ($n = 3$) received four injections of saline every 2 h. After one week, 5-HT, 5-HIAA, and NE were measured by HPLC as described under *Materials and Methods*. Results shown are percentages of the following control group means ± S.E.M. in cortex, hippocampus, striatum, brain stem, and hypothalamus, respectively: 5-HT, 5.6 ± 0.5, 3.6 ± 0.6, 2.0 ± 0.4, 8.8 ± 0.8, and 6.7 ± 0.7 ng/mg protein; 5-HIAA, 2.3 ± 0.1, 3.3 ± 0.2, 2.8 ± 0.4, 7.8 ± 0.4, and 4.6 ± 0.6 ng/mg protein; NE, 4.1 ± 0.9, 3.2 ± 0.6, none detected, 11 ± 1, and 30 ± 3 ng/mg protein. Probabilities indicated in the figure are as follows: *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ different from saline-treated rats.

TABLE 1

Effects of 2'-NH₂-MPTP and 2'-CH₃-MPTP in striatum

Each value represents the mean ± S.E.M. of three rats and is expressed in nanograms per milligram protein.

	DA	DOPAC	HVA
Control	70.4 ± 9.1	17.1 ± 1.6	5.58 ± 0.23
2'-NH ₂ -MPTP (4 × 20 mg/kg)	75.2 ± 3.6	11.3 ± 0.12	5.46 ± 0.35
2'-CH ₃ -MPTP (2 × 20 mg/kg)	85.0 ± 5.0	16.3 ± 1.5	5.62 ± 0.62

vealed significant treatment effects for 5-HT [$F(1,6) = 195$, $p < 0.0001$; $F(1,6) = 63.8$, $p < 0.001$], 5-HIAA [$F(1,5) = 153$, $p < 0.0001$; $F(1,6) = 89.4$, $p < 0.0001$], and NE [$F(1,6) = 201$, $p < 0.0001$; $F(1,6) = 52.2$, $p < 0.001$]. Figure 2 shows that 5-HT, 5-HIAA, and NE concentrations in frontal cortex and hippocampus were depleted to 10 to 20% of control 3 weeks post-treatment. In brain stem, 5-HT [$F(1,5) = 12.4$, $p < 0.05$], 5-HIAA [$F(1,5) = 10.0$, $p < 0.05$], and NE [$F(1,5) = 20.9$, $p < 0.01$] levels were significantly decreased to 70%, 45%, and 55% of control, respectively. Finally, hypothalamic 5-HIAA was reduced to ~65% of control [$F(1,6) = 6.13$, $p < 0.05$]. Concentrations of DA and its metabolites in striatum were not significantly different from those of saline-treated animals following 4×15 mg/kg 2'-NH₂-MPTP (DA = $107 \pm 3\%$; DOPAC = $74 \pm 4\%$; HVA = $90 \pm 4\%$ of control).

These results show that rats are selectively vulnerable to the effects of 2'-NH₂-MPTP versus 2'-CH₃-MPTP. Furthermore, both doses of 2'-NH₂-MPTP induced large losses of cortical and hippocampal serotonin and norepinephrine, and these depletions were sustained for at least 3 weeks.

5-HT Transporter Autoradiography. 5-HT transporter density has been used as a measure of serotonergic nerve terminal integrity (Battaglia, 1990). Quantitative autoradiography was performed using [¹²⁵I]RTI-55 to determine the density of 5-HT uptake sites in frontal cortex, occipital cortex, and striatum following the lower 4×15 mg/kg dose of 2'-NH₂-MPTP. Figure 3 illustrates significant decreases in 5-HT uptake site density in 2'-NH₂-MPTP-treated rats to 10, 15, and 25% of control in frontal cortex [$F(1,5) = 16.27$; $p = 0.002$], occipital cortex [$F(1,5) = 8.92$; $p = 0.503$], and striatum [$F(1,5) = 28.09$; $p = 0.0057$], respectively. These data indicate that decreases in serotonin levels are correlated with a decrease in serotonin transporter density, suggesting degeneration of serotonin axons.

Pharmacologic Challenge with *m*-CPP. To determine whether 5-HT depletion results in a functionally significant effect on the serotonin system, rats were challenged with the serotonin agonist/releaser *m*-CPP 11 days after receiving the lower dose of 2'-NH₂-MPTP. In saline-treated rats, *m*-CPP caused a peak increase in body temperature of $+0.6^\circ\text{C}$ at 30 min postdose (Fig. 4). The peak temperature increase induced by *m*-CPP in rats treated with 4×15 mg/kg 2'-NH₂-MPTP was doubled to $+1.2^\circ\text{C}$ [$F(1,7) = 5.33$; $p = 0.03$; Fig. 4]. The results of this experiment demonstrate that prior treatment with 2'-NH₂-MPTP causes a functional supersensitivity in postsynaptic serotonergic receptors responsible for regulating core body temperature.

Discussion

Prior studies have demonstrated a lack of MPTP-induced dopaminergic neurotoxicity in rats, and the present results indicate a similar lack of dopamine depletion following systemic administration of 2'-CH₃-MPTP. The latter findings appear to contradict earlier work by Sundstrom and Samuelsson (1997); however, these authors only reported an extremely modest (~9%) but statistically significant reduction in striatal dopamine following administration of 2×10 mg/kg 2'-CH₃-MPTP to rats (Sundstrom and Samuelsson, 1997). In contrast to 2'-CH₃-MPTP, rats appear to be highly susceptible to the neurotoxic effects of 2'-NH₂-MPTP at doses

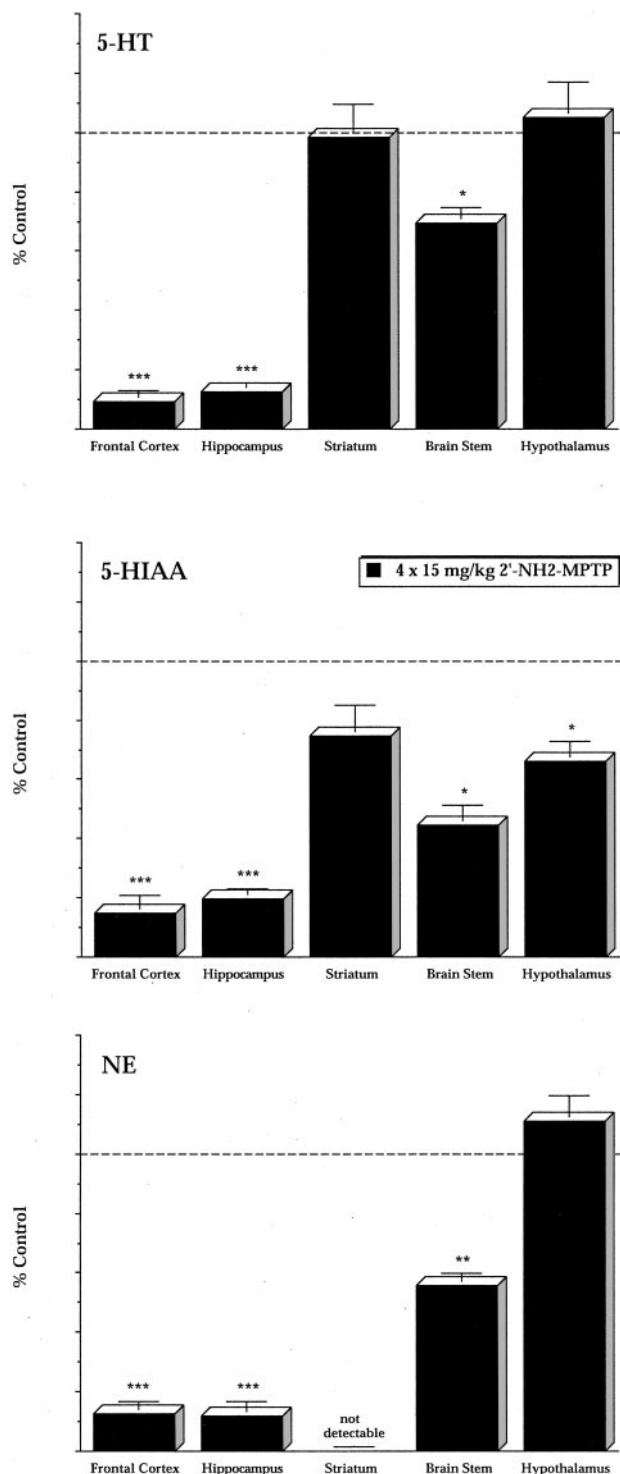


Fig. 2. Changes in 5-HT, 5-HIAA, and NE 3 weeks after 4×15 mg/kg 2'-NH₂-MPTP. Rats were injected with 4×15 mg/kg 2'-NH₂-MPTP at 2-h intervals ($n = 4$), whereas control animals received four similarly timed injections of saline ($n = 4$). Three weeks post-treatment, 5-HT, 5-HIAA, and NE were determined by HPLC as described above and are shown as percentages of the following control group means \pm S.E.M. in cortex, hippocampus, striatum, brain stem, and hypothalamus, respectively: 5-HT, 5.7 ± 0.4 , 3.5 ± 0.4 , 2.2 ± 0.1 , 13 ± 1 , and 6.5 ± 0.1 ng/mg protein; 5-HIAA, 2.6 ± 0.1 , 3.6 ± 0.3 , 3.2 ± 0.4 , 17 ± 3 , and 4.9 ± 0.6 ng/mg protein; NE, 3.2 ± 0.2 , 4.2 ± 0.5 , none detected, 13 ± 1 , and 21 ± 2 ng/mg protein. Probabilities indicated in the figure are: *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ different from saline-treated rats.

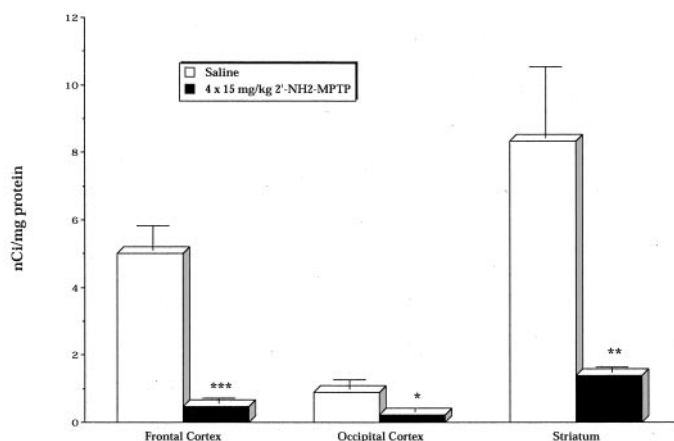


Fig. 3. Autoradiographic determination of 5-HT uptake sites by [¹²⁵I]RTI-55. In rats described in Fig. 2, contralateral brain hemispheres were reserved and sectioned for determination of regional 5-HT uptake site density. Probabilities indicated in the figure are: *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ different from saline-treated rats.

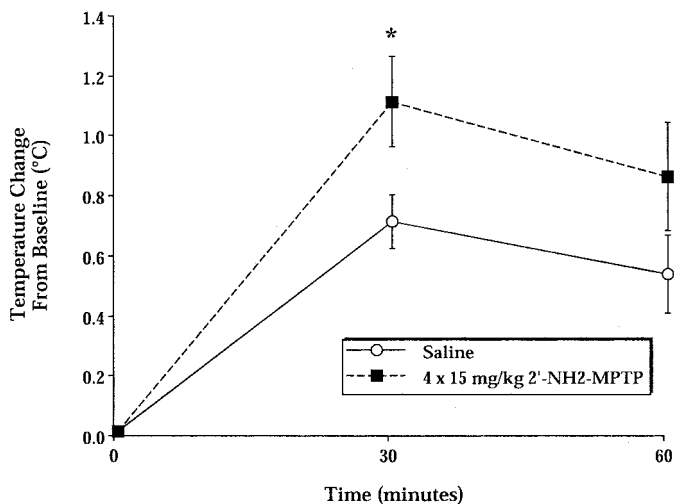


Fig. 4. Effects of 2'-NH₂-MPTP on m-CPP-induced hyperthermia. Rats received either 4 × 15 mg/kg 2'-NH₂-MPTP at 2-h intervals or saline on the same schedule. Eleven days later, rats were acclimated to the testing room temperature of 22.5 ± 0.1°C for at least 1 h. Temperature was measured with a rectal temperature probe once prior to m-CPP administration (1.4 mg/kg) and at 30 and 60 min thereafter. Baseline temperatures were as follows: control = 36.8 ± 0.13°C; 2'-NH₂-MPTP-treated = 36.6 ± 0.13°C.

similar to those producing serotonergic and noradrenergic neurotoxicity in mice. Large depletions in 5-HT and NE were evident at both 1 and 3 weeks post-treatment, particularly in frontal cortex and hippocampus. Although 4 × 20 mg/kg 2'-NH₂-MPTP did not appear to cause an appreciably greater effect on regional 5-HT or NE compared with 4 × 15 mg/kg 2'-NH₂-MPTP, the two doses of 2'-NH₂-MPTP are not strictly comparable because rats from the two experiments were sacrificed at different times.

Decrements in neurotransmitter levels were accompanied by a long-lasting decrease in 5-HT transporter density in cortex and striatum, which is indicative of a loss of serotonin nerve terminals in these brain regions (Battaglia, 1990). The lower dose of 2'-NH₂-MPTP also resulted in a potentiation of the hyperthermic response to m-CPP. 5-HT_{2C} receptors have been shown to mediate m-CPP-induced increases in body temperature in rats (Mazzola-Pomietto et al., 1996). In this

case, the 5-HT_{2C} receptor is most likely up-regulated or functionally supersensitive in response to decreased presynaptic serotonergic input following 2'-NH₂-MPTP administration.

We observed that seizures and death can occur in rats after administration of 2'-NH₂-MPTP. We believe death is due to cardiovascular side effects (Dowling et al., 1987). MPTP and its analogs cause acute release of norepinephrine, dopamine, and/or serotonin (Fuller and Hemrick-Luecke, 1986; Fuller et al., 1988), which can centrally or peripherally mediate elevations in blood pressure and heart rate.

In interpreting the present results in the context of previous findings on MPTP, the following question is apparent: Why are rats sensitive to the serotonin- and norepinephrine-depleting effects of 2'-NH₂-MPTP yet insensitive to neurotoxicity caused by systemically administered MPTP or 2'-CH₃-MPTP? Many previous studies have been directed at investigating the rat's paradoxical lack of sensitivity to MPTP and, as a result, a number of hypotheses regarding this phenomenon have emerged (Johannessen et al., 1985; Kalaria and Harik, 1987; Giovanni et al., 1994a; Russo et al., 1994).

An early hypothesis purported that systemically administered MPTP may be efficiently metabolized by the exceptionally high levels of cerebral microvessel MAO-B found at the blood-brain barrier in rats, thus preventing a critical amount of positively charged MPP⁺ from entering the rat brain (Kalaria and Harik, 1987). However, this same study showed that rats also have the highest cerebral microvessel MAO-A activity among the six species tested; thus, one would expect rats to be equally insensitive to 2'-NH₂-MPTP and 2'-CH₃-MPTP, both of which require oxidation by MAO-A (Kindt et al., 1988; Andrews and Murphy, 1993a). In fact, as shown here, rats are selectively susceptible to the serotonergic and noradrenergic neurotoxicity of 2'-NH₂-MPTP; therefore, the relative activities of MAO-A or -B at the blood-brain barrier seem an unlikely explanation for the differential sensitivity of rats to MPTP and its analogs.

In a series of comparative experiments in rats and mice, Giovanni et al. (1994a) ruled out variations in brain MAO-A or MAO-B activity or in striatal DA transport as possible explanations for the difference in species sensitivity to MPTP. These authors concluded that rat nigrostriatal dopaminergic neurons themselves must be less susceptible to MPTP based on studies using both systemically administered MPTP and intrastriatal MPP⁺.

Clues to possible intrinsic differences in dopamine neurons between rats and mice lie in studies on the vesicular storage of MPP⁺. Early studies by Reinhard et al. (1987) demonstrated that cultured adrenal chromaffin cells accumulate high intracellular concentrations of MPP⁺, yet they are resistant to catecholamine depletions. This resistance was suggested to be due to the ability of chromaffin cells to sequester MPP⁺ in storage granules. Further studies showed that in rats, striatal MPP⁺ was reduced following reserpine treatment to inhibit vesicular transport. In contrast, striatal MPP⁺ levels in mice were unaffected by pretreatment with reserpine (Russo et al., 1994). These authors concluded that in rats, storage vesicles in dopaminergic neurons effectively sequester MPP⁺ from the cytosol, thus shifting the internal cellular balance of MPP⁺ away from mitochondria where oxidative respiration is thought to be deleteriously inhibited by MPP⁺.

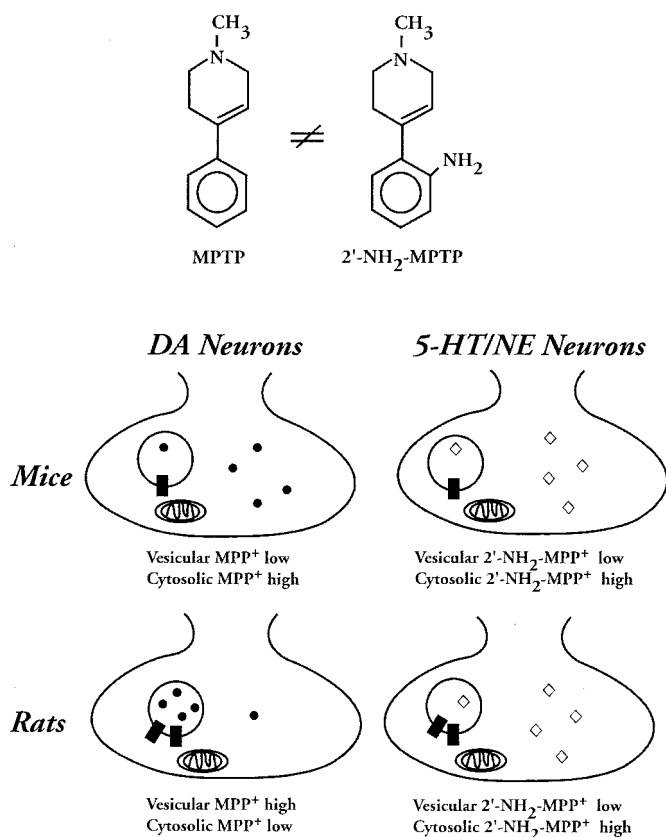


Fig. 5. Hypothesized differences in vesicular sequestration to account for variations in species sensitivity to MPTP versus 2'-NH₂-MPTP. The toxic metabolite of MPTP, MPP⁺, has been shown to be differentially sequestered in synaptic vesicles in rats versus mice. A decrease in the ratio of MPP⁺ in the cytosolic versus vesicular compartments has been theorized to account for the reduced sensitivity of rats to the toxic effects of MPTP. Based on the equipotent effects of 2'-NH₂-MPTP in rats and mice, it appears that 2'-NH₂-MPP⁺ is sequestered to an equivalent extent into vesicles of these two species.

Additional studies by others demonstrated that transfection of MPP⁺-sensitive Chinese hamster ovary cells with cDNA from MPP⁺-resistant PC12 cells produced a population of Chinese hamster ovary cells that were now resistant to MPP⁺ toxicity. This occurred via a reserpine-sensitive mechanism which the authors hypothesized was due to transfection of a vesicular transport system (Liu et al., 1992b). These studies led directly to the cloning of VMAT2 (Liu et al., 1992a).

Recent studies by Sonsalla and coworkers have demonstrated the importance of VMAT2 regarding species sensitivity to MPTP. Pharmacologic inhibition of VMAT2 resulted in enhanced toxicity due to intrastriatal administration of MPP⁺ in rats (Staal and Sonsalla, 2000). Further work has indicated that sequestration of MPP⁺ into rat synaptic vesicles exceeds that of mouse vesicles (Staal et al., 2000). In rat vesicular preparations, the rate of uptake of MPP⁺ by VMAT2 was 2-fold greater than the rate of uptake in mouse preparations, while [³H]dihydrotrabenzazine binding studies with VMAT2 vesicle preparations indicated that B_{max} was also 2-fold greater in rats. These findings provide evidence of a greater density of VMAT2 in rat vesicles, which can partially but not completely account for the species differences in MPTP toxicity.

Our unpublished findings indicate that 2'-NH₂-MPTP is

converted to the corresponding pyridinium by MAO. These data, together with the present findings, suggest that the differences in the sensitivity of rats to MPTP versus 2'-NH₂-MPTP may result from variations in vesicular sequestration of the corresponding pyridiniums (Fig. 5). As discussed, MPP⁺ is sequestered to a greater extent in rats versus mice due to a 2-fold increase in VMAT2 expression in rat vesicles. This leads to decreased MPTP toxicity in rats. With regard to 2'-NH₂-MPTP, differences in the affinity (K_m) of 2'-NH₂-MPP⁺ for VMAT2 between the two species may result in equivalent sequestration of 2'-NH₂-MPP⁺ in rats versus mice. On the other hand, 2'-NH₂-MPP⁺ may not be a good substrate for the VMAT2 in either species, resulting in very little sequestration of 2'-NH₂-MPP⁺ in rats or mice. A direct investigation of the kinetics of uptake of 2'-NH₂-MPP⁺ (especially K_m) into vesicles from rats versus mice is definitely warranted.

Further evidence for the involvement of VMAT2 expression in toxicity comes from studies on VMAT2 knockout mice. These data indicate that VMAT2^(+/-) mice show potentiated MPTP- and methamphetamine-induced dopaminergic neurotoxicity compared with VMAT2^(+/+) mice (Takahashi et al., 1997; Gainetdinov et al., 1998; Fumagalli et al., 1999). Furthermore, data collected on VMAT2 knockdown mice with very low expression of VMAT2 show that these mice are more susceptible to the neurotoxic effects of MPTP than are VMAT2^(+/-) or VMAT2^(+/+) mice (Mooslehner et al., 2001). These data demonstrate that in cases where a neurotoxin is a substrate for VMAT2, vesicular uptake can be an important defense mechanism against neurotoxicity.

However, the conclusions of the present study demonstrate that rats and mice display similar sensitivities to serotonergic and noradrenergic neurotoxicity caused by 2'-NH₂-MPTP. In addition, the effects of 2'-NH₂-MPTP are similarly regionally selective for frontal cortex and hippocampus and, to a lesser extent, brain stem, across the two species. In contrast, rats appear to be resistant to the dopamine-depleting effects of systemically administered 2'-CH₃-MPTP. Further investigation of differential species sensitivity to these neurotoxins may lead to a better understanding of their mechanisms of action and to new information on fundamental differences in the mechanisms of neurodegeneration across monoaminergic neurotransmitter systems.

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Address correspondence to: Anne Milasincic Andrews, 152 Davey Laboratory, Department of Chemistry, Pennsylvania State University, University Park, PA 16802. E-mail: ama11@psu.edu