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Evaluation of the neurotoxicity of *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (*para*-methoxymethamphetamine, PMMA)

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These studies assessed the neurotoxic potential of *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (*para*-methoxymethamphetamine; PMMA), an amphetamine analog that has surfaced in the illicit drug market. Repeated subcutaneous injections of PMMA caused lasting, dose-related reductions in regional brain concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), and in the density of [^{3}H]paroxetine-labelled 5-HT uptake sites. Comparison of the neurotoxic potential of PMMA to that of para-methoxyamphetamine (PMA) and 3,4-methylenedioxymethamphetamine (MDMA) showed that equivalent doses of PMMA and PMA (80 mg/kg) produced comparable depletions of 5-HT, but that these depletions were not as pronounced as those induced by a lower dose of MDMA (20 mg/kg). Striatal DA was not affected on a long-term basis by any of the ring-substituted amphetamines evaluated in this study. These data suggest that PMMA, like PMA and MDMA, produces long-term (possibly neurotoxic) effects on brain serotonin neurons, but that PMMA is less potent than MDMA as a 5-HT neurotoxin. Further, they raise concern over the illicit use of PMMA since humans could be more sensitive than rodents to the 5-HT neurotoxic effects of PMMA and related drugs.

A number of substituted phenylisopropylamines have been identified in the illicit drug market. Of these, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), and 3,4-methylenedioxyamphetamine (MDA) have received considerable scientific attention, largely because of their serotonergic neurotoxic potential[1,3,10,15,17,19,23,27,28,30]. Recently, another amphetamine analog, *N*-methyl-1-(4-methoxyphenyl)-2-aminopropane (*para*-methoxymethamphetamine; PMMA), has surfaced on the illicit market, and in at least one instance, PMMA has been sold as a substitute for MDMA (F. Sapienza, personal communication). Although first synthesized in 1938[8], the pharmacological and toxicological properties of PMMA remain largely uncharacterized. In the only available behavioral study, PMMA was found to be devoid of significant stimulant- or hallucinogen-like activity in drug discrimination studies[5]. A similar behavioral profile has been observed with MDMA[13], although the central effects of PMMA in humans are somewhat different than those of MDMA[26]. Because of the structural relation of PMMA to established neurotoxins such as MDMA and methamphetamine, the present study was undertaken to evaluate PMMA's neurotoxic potential.

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) weighing 180–200 g were housed in individual cages with free access to food and water. Colony rooms were temperature-controlled (22–24°C) and maintained on a 12/12 h light/dark cycle. The drug treatment regimen employed for evaluation of PMMA neurotoxicity consisted of subcutaneous drug injections given twice a day (09.00 and 17.00 h) for 4 consecutive days. To obtain a dose–response relationship, doses of 20, 40, or 80 mg/kg PMMA HCl were tested. In a second experiment, the effects of 80 mg/kg PMMA HCl were compared to those of 80 mg/kg PMA HCl and 20 mg/kg MDMA HCl. The test compounds (ob-

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tained from the National Institute on Drug Abuse, Rockville, MD) were prepare in sterile saline to deliver a volume of 1 ml/kg body weight. One week after the last treatment day, the animals were decapitated, and brains were rapidly removed. The hippocampus, striatum, and frontal cortex were dissected and frozen in liquid nitrogen for subsequent analysis of serotonin (5-HT), dopamine (DA), and their acidic metabolites by high-performance liquid chromatography with electrochemical detection as detailed elsewhere[18]. In other experiments, the binding of [^{3}H]paroxetine (New England Nuclear Corp., Boston, MA) to rat cortical membranes was examined as described by Habert et al.[6] with minor modifications. Tissue was homogenized in 50 volumes of buffer (50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl; pH 7.4) with a polytron homogenizer. The homogenates were centrifuged at $30,000 \times g$ for 10 min. The pellets were resuspended in the same volume of buffer and centrifuged again. The final pellet was resuspended to yield a final concentration of 6 mg tissue/ml. Each sample in the binding assay contained 3 mg tissue. [^{3}H]Paroxetine concentrations ranged from 0.015–0.24 nM. Specific binding was defined as that displaceable by 1 μM citalopram. Samples were incubated for 1 h at 24°C. The reaction was terminated by rapid filtration though Whatman GF/B filters using a Brandel cell harvester. The tubes were rinsed 3 times with Tris buffer, air dried, and placed in scintillation vials to which 10 ml of Ecoscint cocktail was added. Radioactivity was counted in a Beckman scintillation spectrometer. Binding data were analyzed with the EBDA/LIGAND software package (GraphPAD Software, San Diego, CA) and expressed as fmol [^{3}H]paroxetine bound/mg tissue. Data were analyzed using a one-way analysis of variance (ANOVA) with a post-hoc Duncan's multiple range test. The significance level was $P < 0.05$.

Multiple subcutaneous injections of PMMA produced lasting, dose-related reductions of rat brain 5-HT and 5-HIAA concentrations (Table I). The highest test dose of PMMA (80 mg/kg) reduced the level of 5-HT in the hippocampus by 32%, and by 28% in the frontal cortex. Levels of 5-HIAA in the hippocampus and frontal cortex were also significantly reduced by PMMA (Table I). Concentrations of 5-HT in the striatum and hypothalamus of animals treated with 80 mg/kg PMMA were lower than control levels, but these reductions did not attain statistical significance.

The density of [^{3}H]paroxetine-labelled 5-HT uptake sites was also reduced significantly by PMMA (Table II). There was no change in the apparent affinity (K_d) of the binding site.

Rats given the highest doses of PMMA (40 and 80 mg/kg) exhibited clear signs of sympathomimetic stimulation, including salivation, piloerection, lacrimation, and sometimes convulsions. The percent lethality varied between experiments, ranging from 15–43% at the 80 mg/kg dose. The neurotoxic potential of PMMA doses higher than 80 mg/kg was not tested because of the high lethality rate.

Comparison of the neurotoxic potential of PMMA, PMA and MDMA revealed that the 5-HT-depleting effects of 80 mg/kg PMMA were comparable to those of 80 mg/kg PMA, but were generally less than those produced by 20 mg/kg MDMA (Table III). For example, in the hippocampus and frontal cortex, PMMA reduced the levels of 5-HT by 27% and 22%, respec-

TABLE I

Dose–response relationship for the effect of PMMA on regional levels of 5-HT and 5-HIAA

Rats were injected subcutaneously twice daily for 4 days with 0, 20, 40, or 80 mg/kg PMMA and sacrificed one week after the last day of treatment. Data are expressed as the mean ± S.E.M. (ng/mg tissue) for 6–8 animals per group.

	5-HT	*5-HIAA*
Hippocampus		
0	0.25 ± 0.02	0.32 ± 0.03
20	0.26 ± 0.02	0.34 ± 0.02
40	0.19 ± 0.02	0.24 ± 0.03 *
80	0.17 ± 0.02 *	0.17 ± 0.01 **
Striatum		
0	0.34 ± 0.04	0.42 ± 0.03
20	0.34 ± 0.04	0.48 ± 0.05
40	0.25 ± 0.03	0.32 ± 0.03
80	0.27 ± 0.02	0.32 ± 0.03
Frontal Cortex		
0	0.25 + 0.01	0.23 + 0.02
20	0.24 + 0.02	0.21 + 0.01
40	0.19 + 0.02 *	0.17 + 0.01 **
80	0.18 + 0.01 *	0.14 + 0.01 **
Hypothalamus		
0	0.48 ± 0.05	0.42 ± 0.01
20	0.48 ± 0.05	0.38 ± 0.04
40	0.44 ± 0.05	0.34 ± 0.04
80	0.35 ± 0.02	0.33 ± 0.03

* $P < 0.05$ and ** $P < 0.01$ vs. saline-treated controls by one-way ANOVA with a post-hoc Duncan's multiple range test.

TABLE II

Effect of treatment with PMMA on [^{3}H]paroxetine binding sites in rat cortex

Rats were treated as described in Table I with 80 mg/kg PMMA. Data are expressed as the mean ± S.E.M. for 4 independent trials, each of which was conducted on pooled tissue from two rats.

	B_{max} *(fmol / mg tissue)*	K_d *(nM)*
Control	17.4 + 0.7	0.035 + 0.005
PMMA	11.7 + 1.4 *	0.032 + 0.003

* $P < 0.05$ vs. saline-treated controls by a two-tailed Student's *t*-test.

TABLE III

Regional concentrations of 5-HT and 5-HIAA and striatal dopamine one week after treatment with PMMA, PMA, or MDMA

Rats were injected subcutaneously with either PMMA (80 mg/kg), PMA (80 mg/kg) or MDMA (20 mg/kg) twice daily for 4 days and sacrificed one week after the last day of treatment. Data are expressed as the mean ± S.E.M. (ng/mg tissue) for 6–8 animals per group.

	5-HT	*5-HIAA*
Hippocampus		
Saline	0.26 ± 0.01	0.33 ± 0.02
PMMA	0.19 ± 0.01 **	0.21 ± 0.01 **
PMA	0.14 ± 0.02 **	0.15 ± 0.02 **
MDMA	0.13 ± 0.01 **	0.15 ± 0.01 **
Striatum		
Saline	0.30 ± 0.03	0.36 ± 0.02
PMMA	0.26 ± 0.02	0.33 ± 0.03
PMA	0.24 ± 0.02	0.27 ± 0.03 *
MDMA	0.19 ± 0.02	0.24 ± 0.02 **
Frontal Cortex		
Saline	0.40 ± 0.05	0.24 ± 0.03
PMMA	0.31 ± 0.02 *	0.17 ± 0.01 **
PMA	0.28 ± 0.03 **	0.14 ± 0.01 **
MDMA	0.19 ± 0.02 **	0.13 ± 0.01 **
Hypothalamus		
Saline	0.50 ± 0.07	0.37 ± 0.05
PMMA	0.25 ± 0.04 **	0.16 ± 0.02 **
PMA	0.27 ± 0.02 **	0.17 ± 0.01 **
MDMA	0.21 ± 0.03 **	0.17 ± 0.03 **

* $P < 0.05$ and ** $P < 0.01$ vs. saline-treated controls by one-way ANOVA with a post-hoc Duncan's multiple range test.

tively, whereas the 4-fold lower dose of MDMA produced depletions of 50% and 52%, respectively. However, in the hypothalamus, all test compounds caused a relatively equivalent 50% reduction in the concentration of 5-HT. levels of 5-HIAA were also depleted to a greater extent by MDMA than by PMMA (eg. hippocampus: 55% vs. 36% decrease; frontal cortex: 46% vs. 29% decrease). Levels of 5-HT and 5-HIAA in the striatum were lower in the three drug treatment groups, but were not significantly different from control levels. None of the compounds produced persistent alterations in striatal dopamine levels (data not shown).

Assessment of the neurotoxic potential of PMMA indicates that this amphetamine analog produces modest but significant reductions in the concentration of 5-HT and 5-HIAA in several regions of rat brain. In addition, PMMA produces a significant loss of cortical 5-HT uptake sites. Taken together, these findings suggest that PMMA, like MDMA and PMA, possesses serotonergic neurotoxic activity. Of the various regions examined, the striatum appeared to be the least sensitive to PMMA neurotoxicity. The three remaining regions (ie. hippocampus, frontal cortex and hypothalamus) appeared to be comparably affected by PMMA, although the depletion of hypothalamic 5-HT did not achieve statistical significance in one of two experiments (Table I).

The neurotoxic action of PMMA appears to be selective for serotonergic systems since striatal dopamine levels were not reduced on a long-term basis by PMMA treatment. In this regard, PMMA closely resembles MDMA[3,23,28] and *p*-chloroamphetamine (PCA)[7,22], both of which selectively damage serotonergic neurons. In terms of potency, however, the neurotoxic activity of PMMA is considerably lower than that of MDMA since nearly equivalent reductions in 5-HT were produced by PMMA and a 4-fold lower dose of MDMA.

The present results extend the structure–activity relationship for neurotoxic phenylisopropylamines. Ring substitution at the *para* position with halogens (e.g. *p*-chloroamphetamine)[7,22] yields potent serotonergic neurotoxins. The *para*-methoxy-substituted compounds (e.g. PMMA, PMA) also possess 5-HT neurotoxic activity (Table III), but have a reduced potency. Both the *para*-methoxylated and the *para*-halogenated compounds are selectively toxic to serotonergic neurons. By contrast, the unsubstituted compounds d-amphetamine[4] and methamphetamine[9,20,21] possess dopaminergic neurotoxic activity. With regard to the latter compounds, however, *N*-monomethylation appears to confer serotonergic neurotoxic activity since methamphetamine[9,16,21], but not d-amphetamine[16], persistently alters rat brain serotonergic parameters. Notably, *N*-monomethylation seems to have little influence on the potency or the spectrum of neurotoxic activity of the *para*-methoxylated compounds (i.e. PMA and PMMA appear equipotent). Similarly, MDMA and MDA are relatively equipotent with regard to their serotonin-depleting effects[11,24,27,28], as are PCA and its *N*-monomethylated analog[15].

Although not fully characterized, a high rate of lethality was observed after the highest dose of PMMA (e.g. 43% after 80 mg/kg PMMA in one experiment). From these observations, it would appear that the LD_{50} of PMMA is in the range of 80–100 mg/kg. Previous studies have estimated the LD_{50} of PMA in mice to be in the range of 40–70 mg/kg[5,12,14]. Since this dose is only 2–4 times greater than that required to stimulate locomotor activity[5,29], there appears to be a narrow margin between the behaviorally active and lethal dose of PMA. The centrally active dose of PMA in humans is 60–80 mg, and a dose of 150 mg may be fatal in man[25]. Perhaps as a consequence of this narrow safety margin for PMA, a number of overdose deaths were reported after PMA when the compound was illicitly distributed as MDA[2]. Like PMA, an effective dose of 110 mg PMMA in humans produces pro-

found sympathomimetic and cardiovascular stimulation[26]. Given the chemical and pharmacological similarities between PMMA and PMA, concern arises that a similarly narrow margin may exist between the centrally active and lethal dose of PMMA in humans.

In summary, the results of the present study indicate that PMMA causes dose-related reductions in presynaptic serotonergic markers which are most likely reflective of neurotoxicity. The neurotoxic potential of PMMA, coupled with the possibility that there may be a narrow margin between the behaviorally-active and lethal doses of PMMA in humans, raises concern over the recreational use of this compound.

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