Biochemical and Histological Evidence that Methylenedioxymethylamphetamine (MDMA) is Toxic to Neurons in the Rat Brain[1]

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Accepted for publication December 24, 1986

ABSTRACT

(±)-3,4-Methylenedioxymethylamphetamine (MDMA) was administered s.c. to rats (10, 20 or 40 mg/kg b. wt.) and guinea pigs (20 mg/kg) twice a day for 4 days, 2 weeks before decapitation. Norepinephrine, dopamine and serotonin (5-HT) levels were assayed in the hippocampus, hypothalamus, striatum and neocortex. In rats, MDMA produced dose-dependent reductions in 5-HT in all brain regions examined. The highest dose also reduced norepinephrine and/or dopamine in some regions. The 20-mg/kg dose of MDMA depleted 5-HT in all regions of the guinea pig brain assayed. In both species, repeated administration of 20 mg/kg of MDMA reduced the V_{max} but not the K_m of 5-HT uptake 2 weeks after administration. A single 40-mg/kg injection of MDMA depleted 5-HT 2 and 8 weeks after administration to rats in all regions of the brain examined except the hypothalamus. Administration of 80 mg/kg of MDMA twice a day for 2 days to rats depleted striatal 5-HT and dopamine. Brain sections from rats injected with MDMA according to this dosage regimen were stained by the Fink-Heimer method. Degenerating axon terminals and cell bodies were observed in the striatum and somatosensory cortex, respectively. These findings suggest that MDMA is toxic to serotonergic and, to a lesser extent, catecholaminergic neurons. Some neurons that do not contain these transmitters (neocortical neurons) are also affected.

Biochemical analyses indicate that AMPH is toxic to central dopaminergic neurons. Administration of AMPH to rodents either continuously or in a few large doses produces long-term depletions of striatal DA (Wagner *et al.*, 1980; Steranka and Sanders-Bush, 1980), a reduction in tyrosine hydroxylase activity (Ellison *et al.*, 1978) and a loss of DA uptake sites (Wagner *et al.*, 1980; Steranka and Sanders-Bush, 1980). These data, together with supporting histological evidence (Ellison *et al.*, 1978; Ricaurte *et al.*, 1984), strongly suggest that AMPH destroys dopaminergic nerve terminals. The addition of a methyl group to the AMPH molecule, producing MA, does not reduce the toxicity of AMPH. On the contrary, the toxicity of MA to both dopaminergic and/or serotonergic neurons has been documented in a number of species, including rats, cats, monkeys and guinea pigs (Hotchkiss and Gibb, 1980; Ricaurte *et al.*, 1980b; Trulson and Jacobs, 1980; Seiden *et al.*, 1975/76; Wagner *et al.*, 1979).

Recently, it was reported that MDA, a popular illicit drug, is toxic to serotonergic nerve terminals in the rat brain (Ricaurte *et al.*, 1985). Given that the methylation of AMPH does not reduce its neurotoxicity, it seemed pertinent to investigate whether MDMA, a street drug that has also been used by psychiatrists as an adjunct to psychotherapy (U.S. Department of Justice, DEA, 1985), might also have toxic effects on catecholamine and/or 5-HT-containing neurons. Partially out of concern about its potential neurotoxicity, MDMA has been scheduled temporarily as a Schedule I drug by the DEA (U.S. Department of Justice, DEA, 1985). The results of the current study indicate that MDMA is toxic to serotonergic and, to a lesser extent, catecholaminergic neurons in the rat and guinea pig brain.

Methods

Neurochemical experiments. Male Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing 250 to 300 g were housed individually with free access to food (Teklad 4% rat diet) and water. Male Hartley

Received for publication May 14, 1986.

[1] This work was supported by Grant DA-00085 from U.S. Public Health Service, National Institute on Drug Abuse.

[2] Supported by U.S. Public Health Service Training Grant MH-14274. (R. Virus).

[3] Recipient of a Research Scientist Award U.S. Public Health Service Grant MH-10562.

ABBREVIATIONS: AMPH, amphetamine; DA, dopamine; MA, methylamphetamine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethylamphetamine; DEA, Drug Enforcement Administration; 5-HT, serotonin; NE, norepinephrine; 5,6-DHT, 5,6-dihydroxytryptamine; MAO, monoamine oxidase.

guinea pigs (Holtzman Co.) weighing 300 to 400 g were housed in groups of three or four with free access to food (Purina Guinea Pig Chow) and water. The colony rooms for both the rats and the guinea pigs were maintained at 23 ± 2°C under a 12 hr light/dark cycle (lights on 6:00 A.M.–6:00 P.M.).

±-MDMA hydrochloride was obtained from the National Institute of Drug Abuse and administered to rats at doses of 10, 20 or 40 mg/kg, expressed as the salt. The guinea pigs received only the 20-mg/kg dose of MDMA. The control animals of both species received injections of 0.9% saline. In all cases, the injections were made s.c. at a volume of 1 ml/kg b. wt.

In experiments evaluating the effects of multiple injections, rats and guinea pigs were injected twice a day at 9:00 A.M. and 9:00 P.M. for 4 consecutive days and decapitated 2 weeks after the last injection. In another set of experiments, groups of rats were given a single injection of either saline or MDMA at 9:00 A.M. and decapitated 2 or 8 weeks later.

After decapitation, the brains were removed rapidly from the skull and placed on ice. The striatum, hippocampus and hypothalamus were dissected from the rat and guinea pig brains by the method of Heffner *et al.* (1980). The somatosensory cortex was dissected from the rat brains by making three coronal cuts through the brain using razor blades. The first cut was made at the level of the decussation of the anterior commissure. The second and third cuts were made at 1-mm intervals caudal to the first cut. The neocortex in the two sections thus formed was separated from the corpus callosum and trimmed ventrally, approximately 2 mm dorsal to the rhinal fissure and medially, to exclude the cingulate, yielding four dorsolateral strips of somatosensory cortex. All the remaining cortex from the rat brain was pooled to obtain tissue samples referred to as rest of cortex. Whole cortex was obtained from the guinea pig brains by peeling the neocortex away from underlying brain structures.

The tissue samples were stored in liquid nitrogen until assayed for 5-HT, DA and NE by high-performance liquid chromatography with electrochemical detection (Ricaurte *et al.*, 1985). Because levels in control animals are so low, DA was not measured in the hippocampus and somatosensory cortex and NE levels were not measured in the striatum and somatosensory cortex. The data were analyzed by one-way analysis of variance followed by comparisons between the means of the saline control and MDMA-treated rats using Dunnett's *t* test (Winer, 1971).

The synaptosomal uptake of [^{3}H]DA and [^{3}H]-5-HT was measured in rats and guinea pigs 2 weeks after administration of 20 mg/kg of MDMA every 12 hr for 4 days. The uptake of [^{3}H]-5-HT was assessed in hippocampal tissue pooled from two rats and in whole cortex pooled from two guinea pigs. In both species, uptake of [^{3}H]DA was assessed in striatal tissue pooled from two different animals. The tissue samples used in these uptake studies were homogenized immediately after dissection in 50 volumes (w/v) of ice-cold 0.32 M sucrose and centrifuged at 1000 × *g* to obtain a crude synaptosomal preparation. The uptake studies were performed as described in Ricaurte *et al.* (1980b). The data were analyzed using the Lineweaver-Burk double reciprocal transformation and linear regression analysis. The [^{3}H]-5-HT and [^{3}H] DA uptake studies were performed in rats as three separate replicates and were evaluated statistically using Student's *t* tests (Zar, 1984). The uptake studies in guinea pigs were performed only once, in order to confirm the findings made in rats, and so could not be evaluated statistically.

Neurohistological experiments. Adult male rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing approximately 250 g were housed individually as described above except that a slightly different light/dark cycle (lights on 8:00 A.M.–8:00 P.M.) was used. The rats received s.c. injections of either 0.9% saline or 80 mg/kg of MDMA, calculated as the hydrochloride salt and dissolved in saline at a concentration of 40 mg/ml. Some rats received injections of either MDMA or saline twice a day for 2 days. These rats were injected at 10:00 A.M. and 6:00 P.M. on the 1st day and at 10:00 A.M. and 8:00 P.M. on the 2nd day and were perfused 13 to 16 hr after the last injection. Other rats received a single injection of 80 mg/kg of MDMA or saline and were perfused 48 hr later. Lesion experiments have demonstrated that the postoperative period during which the Fink-Heimer method can silver-stain degenerating structures depends on the neural system under study and is transient (Heimer, 1970). In the case of MA-induced neural degeneration, optimal staining occurs within a few days after drug administration. We assumed that the situation would be similar with MDMA. Thus, a high dose of MDMA was administered only once or four times over only 2 days, and short survival times were used.

The rats were perfused with 0.9% saline followed by 10% neutralized formal saline after being anesthetized heavily with sodium pentobarbital (75 mg/kg i.p.). Equal numbers of saline and MDMA-injected rats were perfused on a given day. After perfusion, the brains were removed, placed in perfusion fixative for 5 days and then transferred to perfusion fixative containing 30% (w/v) sucrose. Two days later, 30 μm of frozen coronal brain sections were cut between the levels of A8620 and A5340 of the atlas of König and Klippel (1963) and placed in non-neutralized formal saline. The sections were silver-stained the next day according to procedure I of Fink and Heimer (1967). Brain sections from both control and experimental rats were processed together to equalize variations in staining efficiency across groups. After silver staining, the sections were mounted from alcoholic gelatin onto microscope slides, allowed to air dry and then counterstained with cresyl violet.

All slides were coded and examined by an observer who was unaware of the rat's treatment condition. The striatum of each rat was examined first for the presence or absence of terminal degeneration. The slides were then recoded before the somatosensory cortex was examined in order to exclude any bias in evaluation.

The somatosensory cortex of each rat received a single score of 0 to 5 according to the following treatment scheme: 0) no evidence of terminal, dendritic or cell body degeneration was observed in the somatosensory cortex; 1) some suspicious-looking debris of terminal or dendritic origin was observed. The observer was not certain that the amount of reaction product present was greater than what might be seen in control tissue; 2) debris of terminal or dendritic origin was present consistently enough and in large enough amounts that the observer was confident that it was above control levels; 3) some debris of terminal or dendritic origin was present. At least one obviously reactive cell with fragmented dendrites was observed; 4) the debris appeared to be more common than in category 3. At least one reactive cell was found in almost every section; and 5) a large amount of degenerative debris was present. Multiple reactive cells were observed in almost every section. The degeneration product was present bilaterally. This scheme has been used previously to describe the amount of perikaryal, dendritic and axonal damage observed after administration of MA (Commins and Seiden, 1986).

The histological data were analyzed using the nonparametric Fisher exact probability test (Zar, 1984). In analyzing the striatal data, the proportions of control and experimental subjects that showed terminal degeneration were compared. In analyzing the somatosensory cortical data, the proportions of experimental and control subjects that received a degeneration score greater than or equal to 3 were compared. A degeneration score of 3 was chosen as the dividing point because an animal receiving a score of 3 or greater had degenerating cell perikarya in its somatosensory cortex whereas rats receiving a score lower than 3 did not.

In order to assess the neurochemical deficits produced by the 80 mg/kg x 4 of MDMA dosing regimen, DA and 5-HT levels were measured in the striatum 2 weeks after the last injection of MDMA ($n = 5$) or saline ($n = 6$). The brain dissections and amine assays were performed as described in the section on neurochemical methods. Statistical significance was evaluated using Student's *t* test (Zar, 1984).

RESULTS

Neurochemical Analyses

Repeated injections of MDMA. All 3 doses of MDMA (10, 20 and 40 mg/kg) resulted in significant depletions of 5-HT in

the rat hippocampus, somatosensory cortex and rest of cortex (table 1). Significant 5-HT depletions were observed with only the 20- and 40-mg/kg dose of MDMA in the striatum and 40 mg/kg of MDMA in the hypothalamus (table 1). The effects of MDMA on catecholamine levels (table 2) were modest compared to its effect on 5-HT levels. DA levels in the striatum were reduced by only the 40-mg/kg dose of MDMA. NE levels were decreased in the rest of cortex by both the 20-mg/kg dose of MDMA and the 40-mg/kg dose of MDMA, and in the hippocampus by the 40-mg/kg dose of MDMA.

The 20-mg/kg dose of MDMA produced significant 5-HT depletions in all areas of the guinea pig brain examined (table 3). DA levels, on the other hand, were depleted only in the striatum and NE levels were not significantly affected in any of the brain regions examined (table 3).

Repeated administration of 20 mg/kg of MDMA to rats significantly decreased the V_{max} but not the K_m of the hippocampal high affinity 5-HT uptake system (table 4). The single measurement obtained in guinea pigs support this finding. The V_{max} of [^{3}H]-5-HT uptake into cortical synaptosomes from guinea pigs injected with saline was 1.5 x 10^{-9} mol/g of tissue weight. Administration of MDMA reduced the V_{max} of [^{3}H]-5-HT uptake by 91% to 0.13 x 10^{-9} mol/g of tissue weight. The K_m of [^{3}H]-5-HT uptake was the same in guinea pigs injected with saline (8 x 10^{-7} M) or MDMA (8 x 10^{-7} M). The uptake of [^{3}H]DA into striatal synaptosomes was not affected by MDMA administration in either rats (table 4) or guinea pigs (control and MDMA-injected V_{max} = 1.9 x 10^{-8} mol/gram of tissue weight and K_m = 2 x 10^{-7} M).

A single injection of MDMA. A single injection of 40 mg/kg of MDMA depleted 5-HT significantly at both 2 (table 5) and 8 (table 6) weeks after drug administration in all regions of the rat brain examined with the exception of the hypothalamus. The 20-mg/kg dose of MDMA depleted 5-HT significantly in the somatosensory cortex at both 2 (table 5) and 8 (table 6) weeks after drug administration. The 20-mg/kg dose of MDMA also depleted 5-HT levels significantly in the hippocampus 8 weeks after drug administration (table 6). DA and NE levels were not affected by either dose in any brain region examined (tables 5 and 6).

TABLE 1

Regional brain 5-HT levels in rats 2 weeks after administration of MDMA every 12 hr for 4 consecutive days

Brain Region	MDMA dose	n	5-HT Content[a]	% Decrease from Control Value
	mg/kg		ng/mg tissue	
Striatum	Control	6	0.23 ± 0.022	
	10	6	0.18 ± 0.039	22
	20	6	0.06 ± 0.032	74**
	40	6	0.06 ± 0.034	74**
Hippocampus	Control	6	0.23 ± 0.013	
	10	5	0.16 ± 0.014	30**
	20	5	0.07 ± 0.008	70**
	40	6	0.03 ± 0.003	87**
Somatosensory cortex	Control	6	0.18 ± 0.015	
	10	6	0.10 ± 0.006	44**
	20	6	0.04 ± 0.006	78**
	40	6	0.02 ± 0.006	88**
Rest of cortex	Control	6	0.24 ± 0.018	
	10	5	0.10 ± 0.015	58**
	20	6	0.04 ± 0.018	83**
	40	6	0.02 ± 0.005	92**
Hypothalamus	Control	5	0.61 ± 0.073	
	10	5	0.48 ± 0.115	21
	20	4	0.47 ± 0.058	23
	40	6	0.39 ± 0.044	36**

[a] Values shown are mean ± S.E.M.
** P < .01.

Histological Analyses

Striatum. Argyrophilic deposits indicative of degenerating nerve terminals were present in the striata of five of five rats that were perfused 13 to 16 hr after the last of four 80-mg/kg injections of MDMA, but were not present in the striata of their five saline-injected controls (P = .004) (see fig. 1). However, a single 80-mg/kg injection of MDMA 48 hr before perfusion was not sufficient to produce striatal terminal degeneration in the three rats that received this treatment.

Somatosensory cortex. When stained by the Fink-Heimer method, the primary somatosensory cortex of rats injected either once or 4 times with 80 mg/kg of MDMA contained shrunken, argyrophilic neuronal cell bodies surrounded by what appeared to be fragmented dendrites and degenerating axon terminals (see fig. 2). Most of the degenerating neurons were observed in layers III and IV in a region extending from about one-half to two-thirds of the way between the longitudinal

TABLE 2

Regional brain catecholamine levels in rats 2 weeks after administration of MDMA every 12 hr for 4 consecutive days

Brain Region	MDMA dose	n	NE Content[a]	DA Content[a]
	mg/kg		ng/mg tissue	ng/mg tissue
Striatum	0	6	Not detected	8.7 ± 0.674
	10	6	Not detected	8.6 ± 0.253 (1%)[b]
	20	6	Not detected	8.6 ± 0.270 (1%)
	40	6	Not detected	6.8 ± 0.525 (22%)*
Hippocampus	0	6	0.33 ± 0.019	Not detected
	10	5	0.30 ± 0.024 (9%)[b]	Not detected
	20	5	0.30 ± 0.017 (9%)	Not detected
	40	6	0.24 ± 0.021 (27%)*	Not detected
Rest of cortex	0	6	0.16 ± 0.010	Not detected
	10	5	0.14 ± 0.003 (13%)	Not detected
	20	6	0.13 ± 0.008 (19%)*	Not detected
	40	6	0.13 ± 0.010 (19%)*	Not detected
Hypothalamus	0	5	2.10 ± 0.162	0.18 ± 0.036
	10	5	2.03 ± 0.185 (3%)	0.23 ± 0.049
	20	4	2.40 ± 0.153	0.24 ± 0.054
	40	6	2.10 ± 0.159	0.18 ± 0.033

[a] Values shown are mean ± S.E.M.
[b] Numbers in parentheses are the percentage of decrease from control values.
* P < .05.

TABLE 3

Regional brain 5-HT, NE and DA levels in guinea pigs 2 weeks after administration of 20 mg/kg of MDMA every 12 hr for 4 consecutive days

Brain Region	MDMA Dose	n	5-HT Content[a]	NE Content[a]	DA Content[a]
	mg/kg		ng/mg tissue	ng/mg tissue	ng/mg tissue
Striatum	0	7	0.14 ± 0.023	Not detected	6.3 ± 0.394
	20	8	0.04 ± 0.008* (71%)[b]	Not detected	4.8 ± 0.266** (24%)[b]
Hippocampus	0	7	0.18 ± 0.007	0.32 ± 0.016	Not detected
	20	8	0.07 ± 0.005*** (61%)	0.29 ± 0.013 (9)	Not detected
Whole cortex	0	7	0.13 ± 0.004	0.08 ± 0.004	0.10 ± 0.020
	20	8	0.02 ± 0.001*** (85%)	0.08 ± 0.001	0.06 ± 0.014 (40%)
Hypothalamus	0	7	0.42 ± 0.011	0.93 ± 0.058	0.11 ± 0.013
	20	8	0.17 ± 0.011*** (60%)	0.98 ± 0.044	0.10 ± 0.008 (9%)

[a] Values shown are mean ± S.E.M.
[b] Numbers in parentheses are the percentage of decrease from control values.
* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 4

Kinetic constants of [^{3}H]-5-HT and [^{3}H]DA uptake into synaptosomes 2 weeks after administration of 20 mg/kg of MDMA to rats every 12 hr for 4 consecutive days

Transmitter	Treatment	K_m[a]	V_{max}[a,b]
5-HT	Saline	1.555×10^{-7} M	1.8×10^{-9}
	(n = 3)	$\pm 0.077 \times 10^{-7}$ M	$\pm 0.2 \times 10^{-9}$
	MDMA	1.437×10^{-7} M	0.9×10^{-9}*
	(n = 3)	$\pm 0.070 \times 10^{-7}$ M	$\pm 0.2 \times 10^{-9}$
% Decrease from control			50%
DA	Saline	1.403×10^{-7} M	2.99×10^{-8}
	(n = 3)	$\pm 0.050 \times 10^{-7}$ M	$\pm 0.30 \times 10^{-8}$
	MDMA	1.476×10^{-7} M	2.85×10^{-8}
	(n = 3)	$\pm 0.240 \times 10^{-7}$ M	$\pm 0.30 \times 10^{-8}$

[a] Values shown are mean ± S.E.M.
[b] V_{max} values shown are expressed as moles per gram of tissue weight taken up by synaptosomes.
* $P < .05$.

TABLE 5

Regional brain 5-HT, NE and DA levels in rats 2 weeks after administration of a single dose of MDMA

Brain Region	MDMA dose	n	5-HT Content[a]	NE Content[a]	DA Content[a]
	mg/kg		ng/kg tissue	ng/mg tissue	ng/mg tissue
Striatum	0	6	0.21 ± 0.017	Not detected	7.7 ± 0.538
	20	5	0.17 ± 0.023 (19%)[b]	Not detected	7.7 ± 0.319
	40	4	0.16 ± 0.019 (24%)**	Not detected	8.1 ± 0.496
Hippocampus	0	6	0.18 ± 0.015	0.22 ± 0.020	Not detected
	20	6	0.17 ± 0.013 (16%)	0.28 ± 0.017	Not detected
	40	6	0.14 ± 0.011 (22%)**	0.26 ± 0.020	Not detected
Somatosensory cortex	0	6	0.20 ± 0.002	Not detected	Not detected
	20	6	0.14 ± 0.016 (30%)*	Not detected	Not detected
	40	5	0.12 ± 0.015 (40%)**	Not detected	Not detected
Rest of cortex	0	5	0.20 ± 0.035	0.13 ± 0.014	Not detected
	20	5	0.22 ± 0.022	0.16 ± 0.016	Not detected
	40	4	0.14 ± 0.019 (30%)*	0.14 ± 0.009	Not detected
Hypothalamus	0	5	0.63 ± 0.050	2.1 ± 0.285	0.21 ± 0.024
	20	6	0.67 ± 0.110	2.4 ± 0.112	0.19 ± 0.020 (10%)[b]
	40	5	0.61 ± 0.094 (3%)	2.4 ± 0.140	0.20 ± 0.033 (5%)

[a] Values shown are mean ± S.E.M.
[b] Numbers in parentheses are the percentage of decrease from control values.
* $P < .05$; ** $P < .01$.

fissure and the rhinal sulcus. In the anterior to posterior direction, degenerating cell bodies were observed most reliably around level A5910 of the atlas of König and Klippel (1963) and were consistently found as far posterior as the most posterior level we examined, A5340. Anteriorly, degenerating neurons were consistently observed to level A6360 but were very rarely observed between the level of A6360 and the most anterior level examined, A8620.

All signs of neuronal degeneration, including degenerating cell perikarya, were present in the somatosensory cortex of eight of eight animals injected either once or 4 times with 80 mg/kg of MDMA. Between the levels of A6360 and A5340 of König and Klippel (1963), the average number of degenerating neurons observed per brain section was 0.8 for the single injection (range = 0–2 cells per section) and 3.5 for the multiple injection group (range = 0–9 cells per section). The average degeneration score for rats from both the single and the multiple injection groups considered together was 4.1 (range = 3–5). In contrast, the cortices of the saline-injected rats contained very little degenerative debris. Only a single degenerating neu-

TABLE 6

Regional brain 5-HT, NE and DA levels in rats 8 weeks after administration of a single dose of MDMA

Brain Region	MDMA dose	n	5-HT Content[a]	NE Content[a]	DA Content[a]
	mg/kg		ng/mg tissue	ng/mg tissue	ng/mg tissue
Striatum	0	6	0.19 ± 0.022	Not detected	7.9 ± 0.599
	20	4	0.17 ± 0.011 (11%)[b]	Not detected	8.1 ± 0.170
	40	6	0.12 ± 0.013 (37%)**	Not detected	8.4 ± 0.417
Hippocampus	0	6	0.20 ± 0.019	0.26 ± 0.028	Not detected
	20	4	0.14 ± 0.015 (30%)*	0.24 ± 0.027 (8%)[b]	Not detected
	40	6	0.12 ± 0.012 (40%)**	0.26 ± 0.022	Not detected
Somatosensory cortex	0	6	0.28 ± 0.019	Not detected	Not detected
	20	2	0.20 ± 0.004 (29%)*	Not detected	Not detected
	40	6	0.16 ± 0.021 (43%)**	Not detected	Not detected
Rest of cortex	0	6	0.28 ± 0.038	0.19 ± 0.008	Not detected
	20	4	0.18 ± 0.008 (36%)	0.17 ± 0.011 (11%)	Not detected
	40	6	0.16 ± 0.042 (43%)*	0.19 ± 0.013	Not detected
Hypothalamus	0	6	0.60 ± 0.071	2.1 ± 0.112	0.17 ± 0.019
	20	4	0.68 ± 0.065	2.6 ± 0.261	0.16 ± 0.006
	40	5	0.53 ± 0.054 (12%)	2.0 ± 0.182 (5%)	0.17 ± 0.009

[a] Values shown are mean ± S.E.M.
[b] Numbers in parentheses are the percentage of decrease from control values.
* $P < .05$; ** $P < .01$.

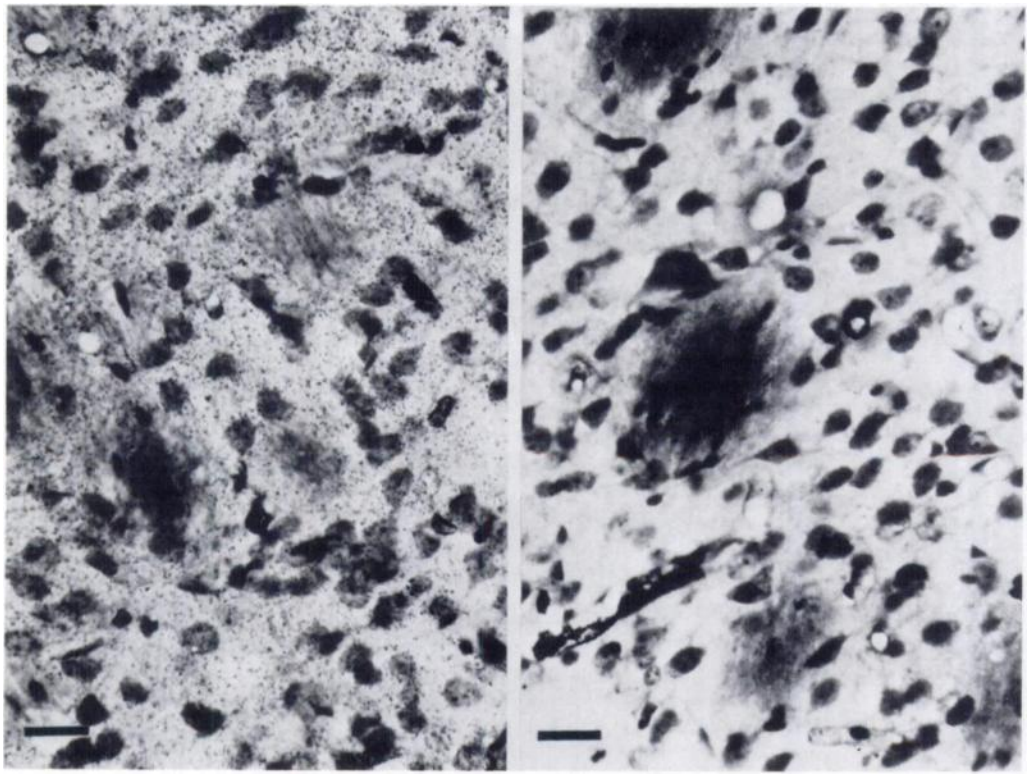

Fig. 1. Left, this photomicrograph shows silver-impregnated degenerating axon terminals (small black dots) in the striatum of a rat injected with MDMA (4 × 80 mg/kg). The rat was perfused approximately 15 hr after the last injection. Fink-Heimer method counterstained with cresyl violet. Right, the striatum of a saline-injected control rat. Note the lack of terminal degeneration. Fink-Heimer method counterstained with cresyl violet. For both photomicrographs, the bar = 25 μm.

ronal cell body was observed in one of the eight controls. The average degeneration score for the control group was 0.9 (range = 0–3). The difference between the degeneration scores received by the experimental and control animal was statistically significant (P = .0008).

Rats that received four 80-mg/kg injections of MDMA 2 weeks before sacrifice had striatal 5-HT levels (0.207 ± 0.025 ng of amine per mg of tissue) which were 56% of control levels (0.371 ± 0.006 ng of amine per mg of tissue; $t = 13.82$, dF = 9, $P < .01$). MDMA administration also reduced striatal DA levels to 67% of control (6.11 ± 0.749 *vs.* 9.11 ± 0.229 ng of amine per mg of tissue; $t = 8.41$, dF = 9, $P < .01$).

Discussion

The results of the present experiments indicate that MDMA was toxic to 5-HT neurons in both rats and guinea pigs. 5-HT depletions were observed in all brain regions examined after multiple injections of MDMA. Repeated administration of MDMA also resulted in a concomitant reduction in the V_{max} of the 5-HT uptake system in rat hippocampus and guinea pig cortex, suggesting an MDMA-induced loss of 5-HT uptake sites in these brain regions. The neurotoxic effect of MDMA is not confined to a multiple injection regimen. Up to 8 weeks after a single injection of MDMA, substantial 5-HT depletions were observed in all brain regions but the hypothalamus. Although repeated administration of the highest dose of MDMA used decreased DA or NE in some brain regions, catecholamine levels were not affected by a single injection of the drug.

In general, the neurotoxic effect of MDMA of 5-HT neurons in the rat was similar to that produced by MA and MDA. Long-term depletions of 5-HT result when moderate doses of any of

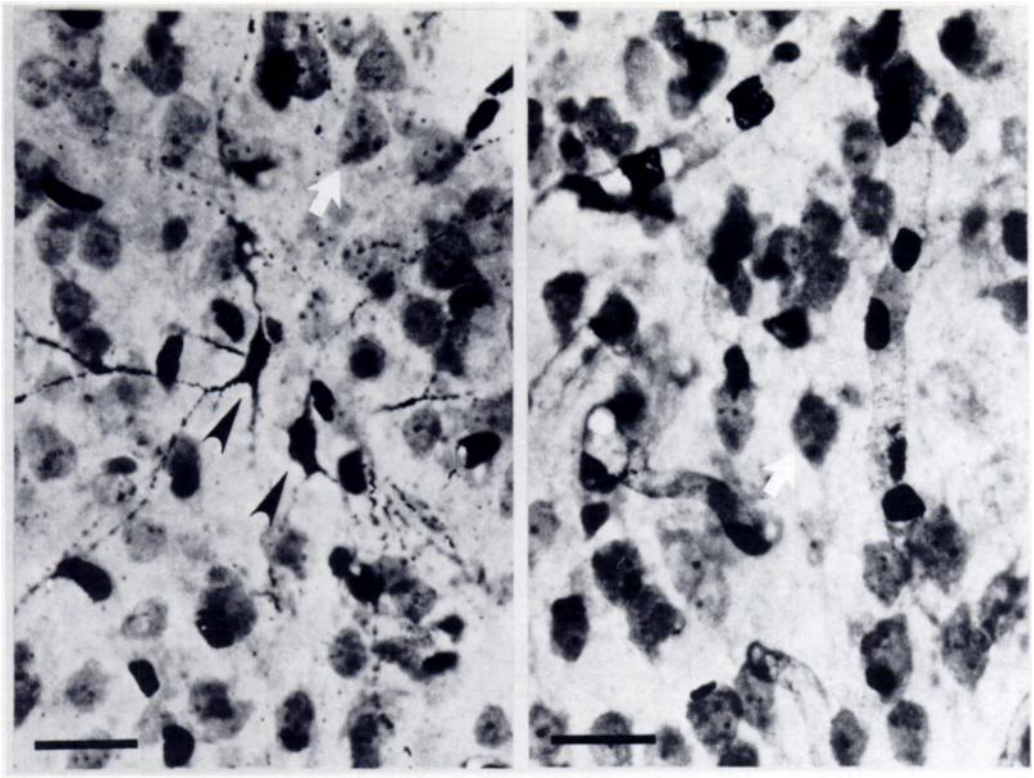

Fig. 2. Left, this photomicrograph shows several degenerating neurons (black arrowheads) in the somatosensory cortex of a rat injected with MDMA (4 × 80 mg/kg). The white arrow indicates an unaffected neuron. The rat was perfused approximately 15 hr after the last injection. Fink-Heimer method counterstained with cresyl violet. Right, the somatosensory cortex of a saline-injected control rat. The white arrow indicates an unaffected neuron. Note the lack of argyrophilic neuronal perikarya. Fink-Heimer method counterstained with cresyl violet. For both photomicrographs, the bar = 25 μm.

these three substituted phenylisopropylamines are administered twice a day for 4 consecutive days (Ricaurte *et al.*, 1980b, 1985). However, MDMA appears to be slightly more effective in depleting 5-HT than either MA or MDA when administered according to this regimen. Ricaurte *et al.* (1985) reported that after administration of 8 x 40 mg/kg of MDA, levels of 5-HT in the striatum and hippocampus were decreased by 58 and 78%, respectively. Similarly, an almost identical treatment regimen using MA (8 x 50 mg/kg) decreased 5-HT levels in the striatum by 48% and 5-HT levels in the whole cortex by 64% (Ricaurte *et al.*, 1980b). In this experiment, the 5-HT depletions observed in the striatum, hippocampus and cortex after treatment with MDMA (8 x 40 mg/kg) exceeded those reported for both MDA and MA. However, a different pattern emerges when 5-HT levels are measured after single injections of each of these drugs. Two weeks following a single injection of 10 mg/kg of MDA, hippocampal levels of 5-HT were reduced by 32% (Ricaurte *et al.*, 1985). The results in table 5 demonstrate that a comparable degree of depletion was achieved with the administration of 40 mg/kg of MDMA. The 5-HT depletions produced in the somatosensory cortex and striatum by 40 mg/kg of MDMA are similar to those produced by a single 100-mg/kg injection of MA (Commins and Seiden, 1986). Thus, the data suggest that, in the rat, a single injection of MDMA is less toxic than a comparable single dose of MDA but more toxic than a comparable single dose of MA.

In the present study, we observed nerve terminal degeneration in the striatum after administration of MDMA according to a dosage regimen that depleted both 5-HT and DA. The Fink-Heimer method does not allow identification of the transmitter contained in the affected nerve terminals, but it seems reasonable to assume that some or all of them contained DA, or possibly 5-HT. Although it has been reported that it is impossible to stain degenerating 5-HT terminals using the Fink-Heimer method (Massari *et al.*, 1978; Harvey *et al.*, 1977), we prefer not to exclude the possibility that we did so in this experiment. Negative results obtained with the Fink-Heimer method must be interpreted cautiously because successful silver impregnation of degenerating terminals depends on the correct combination of numerous variables and is achieved by trial and error (Heimer, 1970). Furthermore, degenerating terminals have been demonstrated in this laboratory using the Fink-Heimer method after a dose of MDA which depleted 5-HT, but not NE or DA (Ricaurte *et al.*, 1985).

Degenerating neuronal cell bodies were observed in the somatosensory cortex after administration of MDMA. Inasmuch as neurons in the neocortex do not contain 5-HT (Azmitia, 1978) or catecholamines (Lindvall and Björklund, 1978), this demonstrates that the effects of MDMA are not confined to these systems. The cell perikarya affected by MDMA appear to be identical to those destroyed by MA (Commins and Seiden, 1986). In the case of both MDMA and MA (Commins and Seiden, 1986; Ricaurte *et al.*, 1980a), almost all of the degenerating neurons were located in layers III and IV of primary somatosensory cortex and were observed most consistently in a restricted region of cortex that extended from approximately one-half to two-thirds of the way between the central longitudinal fissure and the rhinal sulcus.

How far the cortical area affected by MDMA extends in the anterior-posterior plane is not certain because we did not examine the entire cortex for degenerating neurons. In the present study, brain sections from between levels A8620 and A5340 of König and Klippel (1963) were examined. Degenerating neurons were present between levels A6360 and A5340 but were very rarely observed anterior to level A6360. After administration of MA, degenerating neurons were not observed in the cortex for at least 1.0 mm anterior to level A6860, or posterior to level A5150, and thus seemed to be confined to an

area of cortex extending about 1.5 mm in the anterior to posterior direction (Commins and Seiden, 1986). Given the other similarities between the effects of MDMA and MA on somatosensory cortical neurons, it seems likely that the neurons destroyed by MDMA are also localized to a small area of cortex. This regional localization suggests that these neurons probably were not destroyed by a nonspecific effect of MDMA on cerebral blood flow or metabolism.

One might question whether or not the neurons stained with silver by the Fink-Heimer method are actually degenerating. The Fink-Heimer method is widely accepted as a valid technique for staining degenerating axons and terminals (Heimer, 1970). Although it is not usually used to demonstrate degenerating neuronal perikarya, there is no *a priori* reason to assume that it is not also appropriate for this purpose and, indeed, laboratories other than our own have used it to stain degenerating cell bodies (Harvey *et al.*, 1977).

In addition, the morphology of the stained neurons support the supposition that they are dying. Their dendrites appeared to be fragmented and their perikarya were sometimes so shrunken that they were suspended in a hole in the tissue which was presumably created by the perikaryon pulling away from the surrounding neuropil. On occasion, glia have been observed in close association with these silver-stained neurons, suggesting that the neurons were being phagocytized (Ricaurte *et al.*, 1980a).

The mechanism by which AMPH-related compounds exert their neurotoxic effects on 5-HT neurons is not well understood. Studies in this laboratory have shown that after MA administration 5,6-DHT, a serotonergic neurotoxin, is formed in the hippocampus and somatosensory cortex (Axt *et al.*, 1985). Administration of MA causes release of 5-HT (Schmidt and Gibb, 1985). Because MAO is inhibited by MA, 5-HT cannot be degraded as rapidly (Suzuki *et al.*, 1980). We have proposed (Commins and Seiden, 1986) that under these conditions of increased release accompanied by MAO inhibition, 5,6-DHT may be formed by a nonenzymatic oxidative reaction. The 5,6-DHT could be formed either within the terminal or within the synapse and then taken up into the presynaptic terminal where it might cause damage by crosslinking proteins (Rotman *et al.*, 1976). Inasmuch as neocortical neurons do not contain catecholamines (Lindvall and Björklund, 1978) or 5-HT (Azmitia, 1978), it is difficult to understand why some of them are destroyed by MA (Commins and Seiden, 1986) and MDMA. We have hypothesized previously (Commins and Seiden, 1986) that the affected cortical neurons are destroyed by high levels of 5,6-DHT produced by their serotonergic afferents. The specificity of neurotoxins such as 5,6-DHT is dose-related and 5,6-DHT, in particular, is prone to producing nonspecific damage (Baumgarten *et al.*, 1977).

The histological and neurochemical data presented for MDMA are very similar to those obtained with MA. Furthermore, it has been shown that like MA, + MDMA effectively induces 5-HT release in rat brain synaptosomes (Nichols *et al.*, 1982). Although we have not confirmed that 5,6-DHT can be produced after MDMA administration, it is conceivable that this drug produces neurotoxicity in a manner analogous to MA's proposed mechanism of action.

The present study raises the question of whether MDMA is toxic to 5-HT neurons in humans. Because of the differences in species, dose, frequency and route of administration, as well as differences in the way in which rats and humans metabolize AMPHs (Smith and Dring, 1970), it is difficult to extrapolate the present findings to humans. However, if the present study is examined with regard to data obtained with AMPH and MA in both rats and humans, a potentially neurotoxic effect of MDMA in humans is suggested. A minimum dose of 2 mg/kg of AMPH or MA is needed to produce anorexia in the rat (Heffner and Seiden, 1979). Treatment with 10 mg of either AMPH or MA will produce anorexia in humans (Goodman and Gilman, 1958). Assuming an average body weight of 60 kg, the human dose of AMPH or MA required for anorexia is roughly 0.2 mg/kg or 10-fold less than that required for the rat. Application of this 10-fold conversion factor demonstrates that administration of 20 to 40 mg/kg of MDMA to the rat is comparable to the 75 to 150 mg or 1.25- to 2.5-mg/kg dose of MDMA normally ingested by human beings (Greer and Strassman, 1985). If MDMA is administered to humans regularly, it is clear that the toxic potential would be even greater.

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