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Exhibit 10

Hallucinogenic Amphetamine Selectively Destroys Brain Serotonin Nerve Terminals: Neurochemical and Anatomical Evidence

Abstract. (±)-3-4-Methylenedioxyamphetamine (MDA), an amphetamine analogue with hallucinogenic activity, produced selective long-lasting reductions in the level of rat brain serotonin (5HT), the number of 5HT uptake sites and the concentration of 5-hydroxyindoleacetic acid (5HIAA). Morphological studies suggested that these neurochemical deficits were due to 5HT nerve terminal degeneration. These results show that MDA possesses brain 5HT neurotoxic activity and raise the question of whether exposure to MDA and related hallucinogenic amphetamines can produce brain 5HT neurotoxicity in humans.

Keywords: Neurotoxicity - Serotonin - Amphetamines - Hallucinogenic Drugs

(+)-3,4-Methylenedioxyamphetamine (MDA) is a synthetic amphetamine derivative which produces a mixture of psychomotor stimulant and hallucinogenic effects (1). This combination of psychotropic actions may stem from MDA's close structural relationship to both amphetamine, a prototypic stimulant, and mescaline, a well-known hallucinogen. Clinically, MDA has been evaluated as an anorectic, antidepressant and as an adjunct to psychotherapy (2). Although some investigators have advocated that MDA be used to facilitate psychotherapy, MDA has yet to find an accepted place in the medical pharmacopoeia. In contrast, MDA has been a popular illicit drug for over 20 years (3). Despite recognition of MDA's high abuse liability, relatively little research has been done to assess its toxicity. The few studies performed in animals indicate that the toxicity of MDA generally parallels that of amphetamine (4). As such, MDA can produce mydriasis, profuse salivation, tachycardia, hypertension, hyperthermia, convulsions and death. The few studies done in humans suggest that in doses up to 300 mg MDA is free of significant toxicity (2). Higher MDA doses have been associated with near fatal as well as fatal reactions (5). Marked physical exhaustion lasting up to 48 hours after drug ingestion (100-300 mg) has been reported (6).

Amphetamines such as d-methamphetamine and d-amphetamine are toxic to brain dopamine (DA) and serotonin (5HT) neurons (7). This toxicity is manifested by long-lasting reduction in the levels of DA and 5HT and a decreased number of uptake sites in the brain (7). In the case of DA neurons, these deficits have been shown to be the result of DA nerve terminal degeneration (8). In light of these findings and in view of the paucity of information on MDA toxicity, the present study evaluated the DA, 5HT and norepinephrine (NE) neurotoxic potential of MDA. We now present chemical and anatomical evidence of selective brain 5HT nerve terminal degeneration after single or multiple doses of MDA.

We examined the neurotoxic potential of various doses (1.25, 2.5, 5, 10, 20 and 40 mg/kg) of MDA by administering each of these doses subcutaneously to a group

(N=4) of rats every twelve hours for four consecutive days and then assessing the effect of these MDA dosing regimens on brain DA, 5HT and norepinephrine (NE) levels measured two weeks after drug treatment (9). Doses were selected to cover a range known to produce from minimal to maximal behavioral effects in rodents (4,10). Regional brain DA, 5HT and NE level determinations two weeks after drug treatment revealed that MDA produced a large depletion of 5HT in various brain regions without affecting the level of either DA or NE in these same regions (Table 1). The lowest dose of MDA in producing this change was 5 mg/kg. This MDA regimen lowered 5HT levels in the hippocampus and rest of brain but not in the striatum (Table 1). Higher dose regimens reduced 5HT levels in all of the brain regions examined. Of note is that even the highest dose (40 mg/kg) regimen of MDA produced no lethality and that two weeks after drug administration MDA treated rats could not be distinguished from control rats by casual observation.

We examined two other 5HT neuronal markers following MDA administration. Rats were administered 10 mg/kg of MDA for 4 days and two weeks later they were killed for hippocampal ^{3}H-5HT uptake and 5-hydroxyindoleacetic acid (5HIAA) level measurements. Kinetic analysis of ^{3}H-5HT uptake (11) by crude synaptosomal suspensions prepared from the hippocampus of saline- and MDA-treated rats indicated that MDA produced a long-lasting reduction in the V_{max} of ^{3}H-5HT uptake without affecting its K_m (V_{max} in controls: 7479 $\pm$ 678 cpm; in MDA rats: 3265 $\pm$ 408 cpm, difference significant at 0.01 level; K_m in controls 0.12 $\pm$ 0.03 uM; in MDA rats 0.16 $\pm$ 0.04 uM, non-significant difference). This result indicates that MDA reduces the number but not the affinity of synaptosomal 5HT uptake sites. 5HIAA level determinations (12) indicated that MDA also produced a long-lasting reduction in 5HIAA concentration in the hippocampus (5HIAA in control rats: 0.33 $\pm$ 0.03 ug/g; in MDA rats: 0.12 $\pm$ 0.01 ug/g, difference significant at 0.01 level). This finding, along with the observations of

decreased 5HT level and uptake following MDA administration, strongly suggest that MDA is toxic to 5HT neurons.

To confirm this, we looked for evidence of 5HT nerve terminal destruction following MDA administration using the Fink-Heimer method (13) which allows for selective silver-impregnation of degenerating axons and terminals. With this method, degenerating nerve terminals were found in the hippocampus and striatum of all three rats administered MDA (Figure 1). No such terminal degeneration was found in any of the three control rats. Given that the hippocampus and striatum are the same brain regions in which MDA produced selective long-lasting 5HT depletions (Table 1), it seems reasonable to conclude that the degenerating nerve terminals in Figure 1 are serotonergic and that the way in which MDA induces prolonged 5HT neurochemical deficits is by destroying 5HT nerve terminals.

In a final experiment rats were administered 10 mg/kg of MDA every twelve hours for 4, 2, 1 and 0.5 days and killed two weeks later. 5HT level determinations at this time revealed that a single 10 mg/kg injection of MDA (0.5 day regimen) reduced hippocampal 5HT content by 32% and that additional injections led to greater 5HT deficits (Table 2).

Our study raises the question of whether MDA produces 5HT neurotoxicity in humans. Given differences in species, dose, frequency and route of administration, as well as differences in the way in which rats and humans metabolize amphetamine (14), it would be premature to extrapolate our findings to humans. It should also be noted that the doses of MDA required to produce 5HT neurotoxicity in the rat (5-10 mg/kg, Tables 1 and 2) are roughly three to five times higher than those required to produce hallucinogenic effects (approximately 1.5 to 3 mg/kg (1,2). Hence, doses of MDA generally ingested by humans may not be sufficiently high to induce 5HT neurotoxicity, unless humans prove to be more sensitive than rats to the toxic effects of MDA. That this may be the case is suggested by the observation that a 7.5 mg/kg dose of MDA

approaches the lethal dose in humans (5) whereas in rats even a 40 mg/kg regimen did does not produce any lethality (vida supra).

Other ring-substituted amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA), 3,4,5-trimethoxyamphetamine (TMA) and 2,5-dimethoxy-4-methylamphetamine (DOM) are widely abused and possible toxic effects on 5HT neurons of these ring substituted amphetamines need to be evaluated. Such studies should help identify the structural requirements for a ring-substituted amphetamine to produce 5HT neurotoxicity. A better understanding of such structure-activity relations could be of value in suggesting ways in which endogenous substances (e.g. biogenic amines and free phenylethylamines) which are structurally related to MDA and other toxic amphetamines might be modified in vivo into neurotoxic compounds. Such endogenously formed neurotoxins (15) could play a role in the etiology of neurodegenerative disorders involving monoamine-containing neurons in the central nervous system of humans.

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9. Male albino Sprague-Dawley rats weighing approximately 250 grams were obtained from the Holtzman Co. (Madison, WI) and housed singly in suspended wire-mesh cages with free access to food (Purina Rat Chow) and water in a colony room maintained at 23±1 C. (±)-MDA hydrochloride was obtained from the National Institute on Drug Abuse. Its purity was confirmed by means of mass spectroscopic analysis. MDA was administered subcutaneously after being dissolved in sterile 0.9% saline at various desired concentrations. Dose (expressed as the free base) was adjusted by injecting each of these MDA solutions on a 1 ml/kg basis. Control rats were injected with an equal volume of saline. Regional brain DA, 5HT and NE levels were determined by high performance liquid chromatography coupled with electrochemical detection. DA and 5HT were measured according to the method of R. Keller, A. Oke, I. Mefford, R. Adams, Life Sciences 19, 995 (1976), as modified in this laboratory (J. Lucot, J. Horwitz, L.S. Seiden, J. Pharmacol. Exp. Ther. 217, 738 (1981). NE was analyzed using the method of R.S. Fenn, S. Siggia, D.J. Curran, Analyt. Chem. 50 (8), 1067 (1978).

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11. ^{3}H-5HT uptake by crude synaptosomal hippocampal suspensions was measured and kinetically analyzed as described previously; G.A. Ricaurte, L.S. Seiden, C.R. Schuster, Brain Res. 193, 153 (1980). The only important difference was that hippocampal tissue was homogenized in 25 rather than 50 volumes (w/v) of 0.32 M sucrose.

12. 5HIAA levels were measured using reverse-phase high performance liquid chromatographic procedures as outlined by C. Kotake, G. Vosmer, T. Heffner and L. Seiden. Pharmacol. Biochem. Behav. 22, 85 (1985).

13. Prior to doing terminal degeneration studies in MDA treated rats the ability of the Fink-Heimer method to demonstrate 5HT terminal degeneration was assessed. 75 micrograms of 5,7,DHT were dissolved in 0.9% NaCl containing ascorbic acid and injected into the left lateral ventricle. 18 hours later terminal degeneration was found in both the rat hippocampus and striatum. This short survival time seems critical as terminal degeneration is not observed after longer survival times. R.P. Fink and L. Heimer, Brain Res. 4, 369 (1967); V.J. Massari, Y. Tizabi, E. Sanders-Bush, Neuropharmacology 17, 54 (1978).

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Table 1

Regional Brain Monoamines Two Weeks After Various Doses of MDA[1]

	Striatum		Hippocampus		Rest of Brain		
	DA[2]	5HT	NE	5HT	DA	NE	5HT
Treatment							
Saline	11.6±0.3	0.43±-0.05	0.34±0.02	0.41±0.02	0.19±0.01	0.46±.01	0.32±0.04
MDA							
1.25 mg/kg	10.6±0.4	0.42±0.02	NM[3]	0.39±0.03	0.17±0.01	NM	0.29±0.01
2.5 mg/kg	11.7±0.4	0.37±0.04	NM	0.40±0.02	0.18±0.01	NM	0.34±0.03
5 mg/kg	12.4±0.7	0.36±0.04	NM	0.28±0.04*	0.18±0.01	NM	0.23±0.03*
10 mg/kg	11.5±0.6	0.18±0.05*	0.31±0.06	0.16±0.05*	0.18±0.02	0.46±0.04	0.19±0.02*
20 mg/kg	10.6±0.4	0.14±0.02*	0.38±0.01	0.10±0.01*	0.17±0.02	0.47±0.01	0.16±0.02*
40 mg/kg	10.8±0.5	0.11±0.01*	0.40±0.02	0.10±0.01	0.18±0.02	0.43±0.02	0.15±0.02*

[1] Each MDA dose was administered approximately every 12 hours for 4 consecutive days.

[2] Values represent the mean ± S.E.M. expressed in ug/g tissue (N=4).

[3] Not measured since these MDA doses produced little or no effect on 5HT.

* $p < 0.05$, two-tailed student's t-test.

Table 2

Hippocampal 5HT Content Two Weeks After
Various 10 mg/kg Regimens of MDA

Regimen Duration	Hippocampal 5HT	% Decrease
Control	0.41 ± 0.02	-
0.5 day	0.28 ± 0.04*	32
1 day	0.17 ± 0.01*	59
2 days	0.12 ± 0.01*	74
4 days	0.10 ± 0.01*	76

* Significantly different from saline control ($p < 0.05$).

Fig. 1 Legend. Coronal silver-stained sections through the striatum of (A) control rat and (B) a rat administered 10 mg/kg of MDA subcutaneously twice, 12 hours apart. Fink-Heimer method (Procedure I) with cresyl-violet counter-stain. 18 hour survival.