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PERSISTENT NEUROCHEMICAL AND STRUCTURAL CHANGES IN RAT BRAIN AFTER ORAL ADMINISTRATION OF MDMA

S. F. Ali, A. C. Scallet, G. D. Newport,
G. W. Lipe, R. R. Holson and W. Slikker Jr.

Division of Reproductive & Developmental Toxicology
National Center for Toxicological Research
Jefferson, AR 72079-9502 U.S.A.

Summary

MDMA has been reported to produce serotonergic neurotoxicity in rats and monkeys. Most of these studies were conducted at one, two or four weeks after dosing. In order to determine the persistence and potential for recovery from MDMA effects, we performed neurochemical and immunohistochemical assessments at four months after dosing. Adult female CD rats were gavaged with 40 mg/kg MDMA or saline two times a day for four days. Animals were sacrificed by decapitation or by perfusion 120 days after the last treatment and their brains were dissected into different regions for neurochemical and neurohistological evaluation. Brain regions were analyzed for levels of dopamine, serotonin (5-HT) and their metabolites by HPLC with electrochemical detection. MDMA produced significant depletion of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex. In the hippocampus, the 5-HT concentration was also significantly decreased and 5-HIAA concentration showed the same but nonsignificant trend. Neurohistological observation with a specific stain for serotonergic cells and fibers indicated changes in the appearance, but not number, of brain stem cell bodies in the MDMA-treated animals as compared to controls. These data suggest that MDMA produced marked effects on serotonergic axons which are persistent in the frontal cortex and hippocampus of female rats.

Introduction

Methylenedioxymethamphetamine (MDMA) is an amphetamine analog which is recognized as a drug of abuse known as Ecstasy and has also been used by health professionals in psychotherapy (Greer, 1985). Recently investigators have demonstrated that MDMA can be self-administered by both monkey

(Beardsley et al., 1988) and baboons (Lamb and Griffiths, 1987). Several investigators have reported that 5-HT and 5-HIAA concentrations are significantly reduced in the rat brain (Ali et al., 1987; Finnegan et al., 1988; Schmidt, 1987; Schmidt et al., 1986; Slikker et al., 1988) and in monkey brain (Kleven et al., 1987; Slikker et al., 1988) after acute single or multiple injections of MDMA. In most of these studies, the subcutaneous (sc) route of administration was used except for Slikker et al. (1988) and Finnegan et al. (1988) in which the investigator orally gavaged the animal with MDMA. The terminal points in these studies were varied from a few hours up to four weeks after MDMA administration. The only report available on the recovery process from MDMA serotonergic neurotoxicity (Battaglia et al., 1988) showed recovery in 5-HT uptake sites 6 and 12 months after MDMA exposure in male rats. These researchers suggested that there may be a fast initial rate of recovery of 5-HT uptake sites occuring between 18 hours and 4 weeks which is followed by a slow rate of recovery which occurs between 4 weeks and 12 months. These data also suggested that more than 6 months are required for a complete recovery of 5-HT uptake sites to control levels (Battaglia et al., 1988). However, no data has yet been reported to evaluate whether these changes in uptake sites are accompanied by altered concentrations of neurotransmitters. The present study was designed to determine if oral administration of MDMA would result in neurochemical and immunohistochemical alterations four months after treatment of the female rat.

Materials And Methods

Twenty-four female CD rats, weighing 200 $\pm$ 20g were used in this study. Animals were housed two to a cage and maintained on a 12 hour light/dark

cycle in a controlled-environment room (20 ± 2°C, 50 ± 10% relative humidity). Food (Purina Laboratory Chow, St. Louis, MO) and tap water were available ad libitum. A racemic mixture of (±) MDMA hydrochloride was kindly supplied by the National Institute on Drug Abuse, Washington, D.C. The structure was confirmed by chemical ionization mass spectrometry at the NCTR.

Animals were divided into two groups of 12 rats. Group 1 (N=12) received MDMA orally at the dose of 40 mg/kg twice daily (approximately 12 hours apart) for four consecutive days (total of 8 doses). Group 2 received saline solution at the same volume (2 ml/kg) two times a day for four days. Based on our previous data, (Slikker et al., 1988) this regimen of MDMA treatment produced neurochemical and neurohistological alteration in different regions of the brain one month after administration. At 120 days after the last dose of MDMA, 6-8 animals from each group were killed by decapitation; brains were removed quickly and dissected following the guidelines of Glowinski and Iversen (1966). Tissues were placed on dry ice and stored at -70°C until the time of assay. 5-HT, dopamine and their metabolites were resolved by high performance liquid chromatography (HPLC) and quantified by electrochemical detection (Ali et al., 1986). Briefly, each individual region of the brain was weighed and placed in a measured volume (10:1, v/w) of 0.2 N perchloric acid containing 250 ng/ml of the 3,4-dihydroxybenzylamine (DHBA) as internal standard. Brain tissue was then disrupted by ultrasonication and centrifuged (1000xg, 5 min), and 150 ul of the supernatant was removed and filtered through a 0.2 Mm microfilter (MF-1 microcentrifuge filter, Bioanalytic System (BAS), W. Lafayette, IN). Aliquots of 25 ul, representing 10 mg of brain tissue, were injected onto

the HPLC system for separation of the neurotransmitters and their metabolites.

In order to examine the 5-HT system for structural alterations, 2 rats from each group were perfused and the brains were processed for 5-HT immunohistochemical staining. Animals were anesthetized with 60 mg/kg of sodium pentobarbital and sacrificed by fixation with 10% phosphate-buffered formalin perfused through the ascending aorta. Brains were removed the same day as sacrifice and stored overnight in fixative. To eliminate possible effects of length of storage of brains in fixative, all rats of an experiment were sacrificed on the same day, and their brains were sectioned the following day utilizing a Virbrotome (Technical Products, Inc., St. Louis, Missouri). Thirty micron sections were collected in 0.1 M phosphate buffer (pH 7.4) and stored in 24-well plastic tissue culture dishes (Costar, Inc., Cambridge, Massachusetts) in either this same buffer (for immunohistochemistry as described by the methods of Sternberger (1982) or in 10% phosphate-buffered formalin (for the degeneration-specific silver stain described by Nadler and Evenson (1983) until histological processing within the next two weeks. For immunohistochemical stains, primary polyclonal antibodies directed against 5-HT (Incstar Corp., Stillwater, Minnesota) were employed in the present study. Standard primary antibody dilution of 1:500 in an antibody diluent consisting of 2% normal goat serum, 0.3% triton X-100, 0.02% sodium azide, and 0.9% sodium chloride are stored in glass scintillation vials at 5°C. Antisera was vortexed and aliquoted in 1.0 ml volumes to stain the desired number of sections. A large number of sections (up to 24 in 12 vials) were incubated in aliquot of 5-HT antisera. Following an overnight incubation in primary antibody at

5°C, sections are placed (using a glass transfer pipette with the tip melted into a small hook) into the numbered compartments of the staining basket while it is immersed in a tray of 0.1 M phosphate buffer. The basket is placed into a secondary antibody (goat anti-rabbit IgG, diluted 1:100 in antibody diluent, Gibco Laboratories, Grand Island, NY) for one hour at room temperature. After another three 5-minute changes of buffer, the sections are transferred into a 24-well culture dish and incubated for one hour, with each well containing one section in 0.2 ml of peroxidase-antiperoxidase complex (Sternberger-Meyer Immunocytochemicals, Inc., Jarrettsville, Maryland) diluted 1:100 in 0.1 M phosphate buffer. The sections are then returned to the staining basket for another three buffer rinses, and the peroxidase is visualized by enzymatic reaction for about 10 minutes with a solution of 0.05% diaminobenzidine (Sigma Chemical Co., St. Louis, Missouri) in buffer, which is filtered and made 0.1% in hydrogen peroxide just prior to use. Sections are then mounted by drying onto gelatin-coated slides, rehydrated for 30 seconds in distilled water, counterstained (3 minutes in 0.02% cresyl violet), dehydrated through graded ethanols to xylene, and coverslipped with permount. Qualitative estimation of stained cell bodies were made under a microscope by an observer blind to the group identification of the sections.

Statistical analysis of the neurochemistry data were evaluated using analysis of variance followed by Duncan's multiple-range test (Duncan, 1959).

Results And Discussion

Oral administration of MDMA produced significant reductions of 5-HT and 5-HIAA in frontal cortex four months after the last dose (Fig. 1). In the

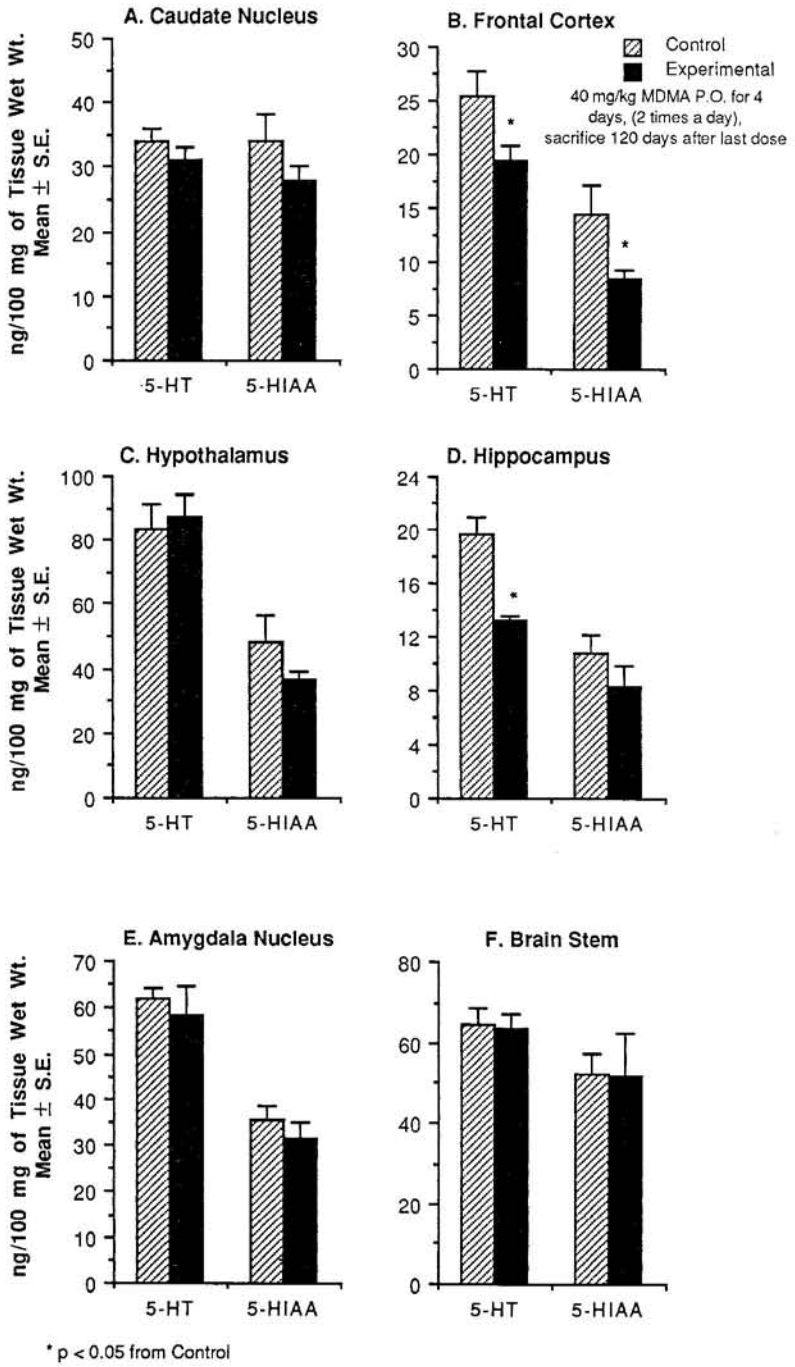

Figure 1. : Concentrations of 5-HT and 5-HIAA expressed as ng/100mg wet weight (mean ± SEM) of tissue in different regions of female rat brain after oral administration of MDMA.

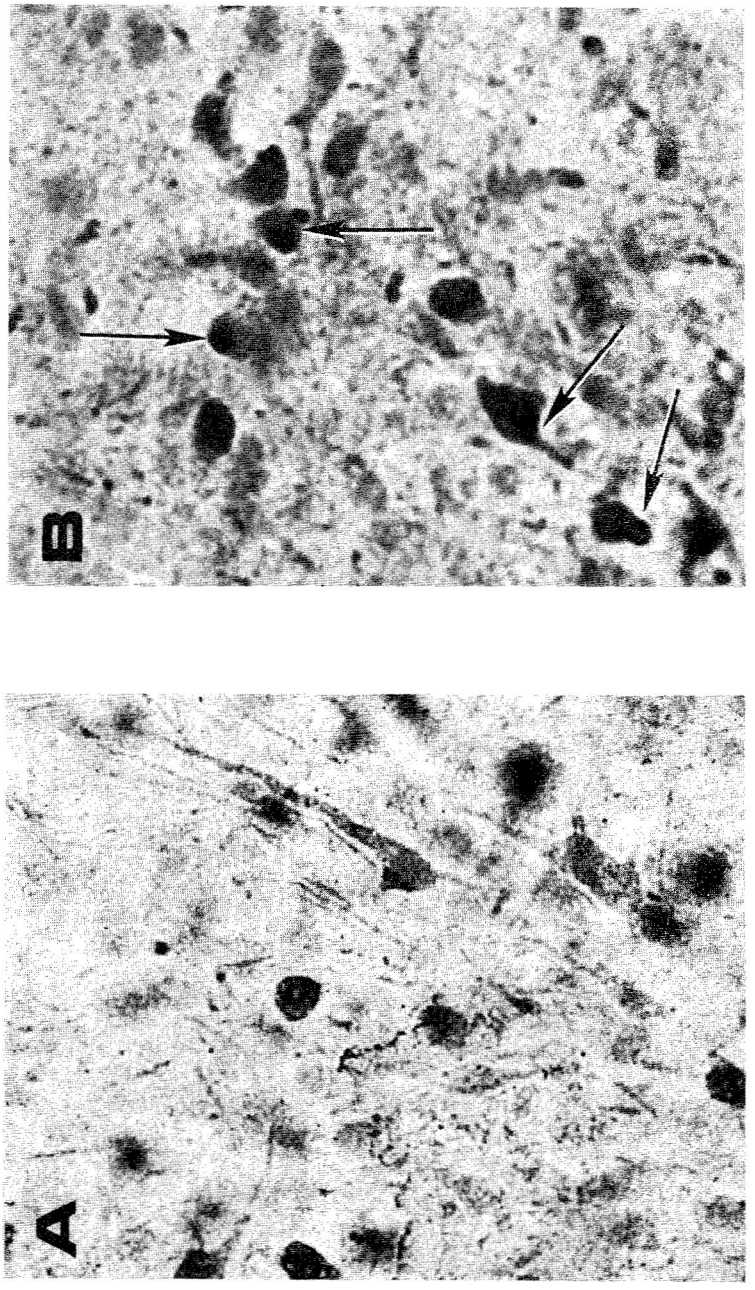

Figure 2. (A). Serotonin-immunoreactive cell-bodies in the dorsal raphe nucleus of the brain stem of a control rat, pretreated one hour before sacrifice with 100mg/Kg of *l*-tryptophan. (B). Serotonin-immunoreactive cell-bodies in the dorsal raphe nucleus of the brain stem of a rat treated 120 days previously with MDMA (and pretreated with 100mg/Kg of *l*-tryptophan one hour before sacrifice). Note the presence of dense inclusion bodies (arrows) in the cytoplasm of these cells and that no such similar inclusion bodies were visible in vehicle (control) treated serotonergic cells (see Figure 2A).

hippocampus, concentrations of 5-HT were significantly decreased whereas 5-HIAA levels showed a similar, if nonsignificant, trend (Fig. 1). These data demonstrate that the effects of orally administered MDMA are long lasting and can be observed for up to four months in the female rat. The frontal cortex and hippocampus were more sensitive to this effect than the caudate nucleus, hypothalamus, amygdala or brainstem.

Recently, we have shown (Slikker et al., 1988) that the same oral dose as used in this study (40 mg/kg) significantly decreased the concentrations of both 5-HT and 5-HIAA in frontal cortex, hypothalamus and hippocampus but only 5-HT in brain stem two weeks after dosing. Together, these studies suggest that the effect produced by MDMA on 5-HT concentrations recovers more slowly for the frontal cortex and hippocampus as compared to the other brain regions. Finnegan et al. (1988) have recently shown that the reduction of 5-HT in hippocampus can be observed with even lower doses of MDMA given either po or sc with the effects determined two weeks after the last dose of MDMA.

Levels of dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid in several brain areas were not significantly altered four months after MDMA exposure (data not shown). In our previous studies (Slikker et al., 1988) oral administration of MDMA (80 mg/kg, p.o.) produced significant depletions of DA in caudate nucleus at two weeks. Finnegan et al. (1988) have also reported a similar depletion at two weeks after oral or sc administration of MDMA. Since we did not find any changes in dopamine four weeks after 80 mg/kg MDMA in our previous study (Slikker et al., 1988) nor any dopamine changes at four months in the present study, there might be two possible explanations. First, the acute effect of MDMA

on serotonergic system is so great that it may also mediate an indirect transient effect on dopamine through serotonergic receptors on dopamine neurons. Alternatively, MDMA has a short term effect on the dopaminergic system first, which in turn mediates the greater effect on the serotonergic system (Gibb et al., 1987).

Neurohistological observation with an antibody to serotonin shows the serotonergic cell bodies in the nucleus raphe of treated and control rats (Fig. 2 A & B). While no apparent differences in cell body density were revealed (Figure 2A vs 2B), a number of inclusion bodies were present in cells of treated but not control rats, suggesting that some abnormalities were present (Fig. 2B).

It is interesting to note that the greatest loss of 5-HT and 5-HIAA concentrations occurred in those areas containing serotonergic axons and nerve terminals while the areas supporting cell bodies were either unaffected or minimally affected by MDMA treatment. Therefore, the data suggests that MDMA predominantly affects the serotonergic nerve terminals rather than cell bodies (Scallet et al., 1988; Slikker et al., 1988). MDMA has been shown to decrease the 5-HT uptake site (Battaglia et al., 1988) while no effect was observed on serotonergic receptor binding sites (Ali et al., 1987). These data also suggest that the site of action of MDMA is predominately serotonergic nerve terminals and axons rather than serotonergic cell bodies. Further studies are under way to determine the maximum duration of MDMA effects on serotonergic terminals and axons, and the minimum dose required to produce these neurotoxicological effects.

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