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MDMA Transiently Alters Biogenic Amines and Metabolites in Mouse Brain and Heart

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STEELE, T. D., D. E. NICHOLS AND G. K. W. YIM. *MDMA transiently alters biogenic amines and metabolites in mouse brain and heart.* PHARMACOL BIOCHEM BEHAV **34**(2) 223-227, 1989.—(±)-3,4-Methylenedioxymethamphetamine (MDMA) (10, 20, and 40 mg/kg) was administered to male CF-1 mice which were sacrificed 3, 6, or 24 hours posttreatment for analysis of brain and cardiac biogenic amines and metabolites. In contrast to reported effects of MDMA in the rat, the highest dose of MDMA transiently elevated mouse brain 5-hydroxytryptamine (5-HT) 3 hours following drug treatment. Levels of dopamine were not significantly affected. 5-Hydroxyindoleacetic acid and dihydroxyphenylacetic acid were significantly lowered by MDMA at the two early time points. The highest dose of MDMA produced a transient depletion of norepinephrine in mouse brain and heart tissue. Only the effects of MDMA on cardiac norepinephrine were prevented by pretreatment of animals with desipramine. A regimen consisting of four daily doses of 40 mg/kg MDMA only produced significant declines in 5-HIAA, dopamine and homovanillic acid levels one week following the last dose. These data confirm previous reports that mice are resistant to the neurotoxic effects of MDMA suggesting that a species variation in response to MDMA exists.

MDMA Biogenic amines Neurotoxicity

A vast array of evidence has recently accumulated which documents the neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) in rats (2, 7, 13, 16, 17, 21–23), guinea pigs (1,7) and monkeys (15). A rapid, dramatic depletion of brain 5-hydroxytryptamine (5-HT) and its acidic metabolite 5-hydroxyindoleacetic acid (5-HIAA) occurs shortly following treatment of rats with a single acute dose of MDMA. Depletion of rat brain 5-HT has been reported to persist for up to two weeks following a single drug administration (7,23) or for up to 110 days following a subacute dosing regimen (23). Decreases in rat brain tryptophan hydroxylase activity (21,23), the capacity of synaptosomes prepared from MDMA-treated rats to accumulate radiolabelled 5-HT (7,16) and a decrease in [^{3}H]-paroxetine binding sites (1) further reflect deficits in serotonergic function in these animals.

The evidence accumulated in various species on these neurotoxic effects of MDMA suggest that use of this compound by humans may have deleterious consequences, which diminishes its potential as a useful psychotherapeutic agent. While studies in primates have suggested that doses reasonably comparable to those used by humans induce changes suggestive of neurotoxicity (15), human cerebrospinal fluid levels of monoamine metabolites were not significantly altered from the norm in frequent users of MDMA (14). The question of whether such markers in humans accurately reflect brain alterations similar to those observed in the primate is a difficult one, but leaves open the possibility that species differences in response to MDMA may exist. Indeed, Stone *et al.* (22) have recently reported that the mouse does not present signs of neurotoxicity similar to those apparent in the rat following administration of MDMA. Differences between the mouse and rat in susceptibility to the long-term changes in serotonergic parameters induced by para-chloroamphetamine (PCA) (19) and fenfluramine (20) have also been demonstrated. These potential species variations are opposite to those observed with the neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which destroys dopamine neurons in the mouse but not the rat (4,6).

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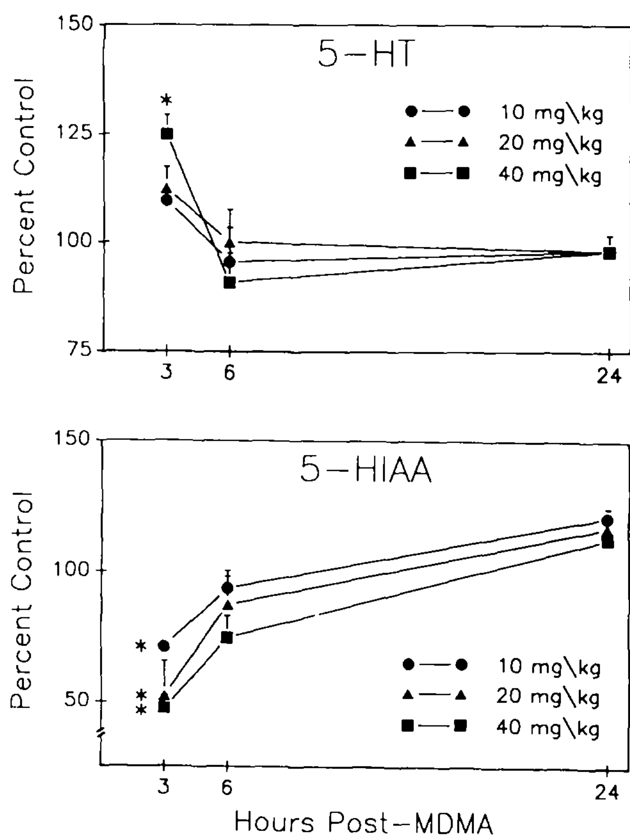

FIG. 1. Effect of acute administration of MDMA on mouse brain levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). Groups of six male CF-1 mice received a single IP injection of different doses of (±)-MDMA HCl and were sacrificed at the indicated time points posttreatment. Shown are the means ± S.E.M. expressed as percent control. $^{*}p<0.05$ vs. saline-treated controls for each time point by two-way analysis of variance.

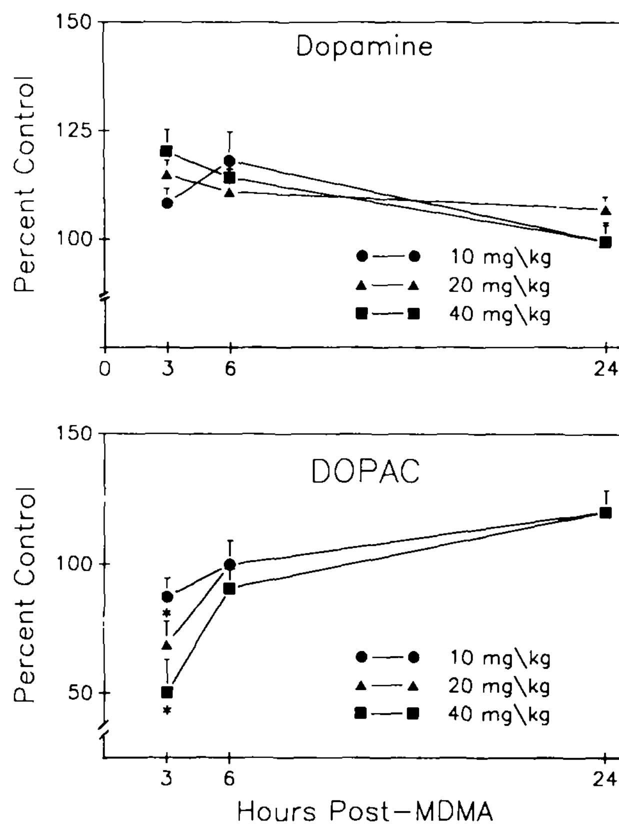

FIG. 2. Effect of acute administration of MDMA on mouse brain levels of dopamine and dihydroxyphenylacetic acid (DOPAC). Experimental conditions and statistical analyses are described in Fig. 1.

Studying species variations in response to toxic agents provides a useful tool for determining the basic mechanism underlying the toxicity. In the studies described here, a detailed assessment of the effects of MDMA on mouse brain biogenic amines and metabolites was conducted to determine if any striking differences in neuronal markers would provide insight into the basis for the species difference. It was also of interest to determine whether MDMA alters cardiac norepinephrine levels, an effect observed with MPTP in rats and mice (11). Similar cardiac effects of MDMA in rats have not been reported, but are of interest in view of the reported deaths potentially attributable to MDMA-induced cardiac arrhythmias (8).

METHOD

Drugs and Reagents

Racemic 3,4-methylenedioxymethamphetamine hydrochloride was synthesized by the method of Braun *et al.* (5). Purity of the compound was verified by elemental analysis, and standard chromatographic and spectroscopic methods. Acid-washed alumina was purchased from Bioanalytical Systems (BAS, West Lafayette, IN); octyl sodium sulfate (OSS) was obtained from Eastman-Kodak (Rochester, NY). Standards for HPLC-EC were purchased from Sigma Chemical Co. (St. Louis, MO); desipramine HCl was obtained from Ciba-Geigy; and HPLC grade methanol and acetonitrile were from Fischer Scientific (Fair Lawn, NJ). All other reagents were of analytical purity.

Animals and Dosing

Male CF-1 mice weighing 25–30 grams were obtained from Harlan Industries (Indianapolis, IN). Animals were housed 8–10 per cage on a 12/12 hour light cycle with food and water available ad lib.

For the time-course and dose-response experiments, animals were randomly assigned to one of four treatment groups (n = 6) for each time point. Time-course and dose-response assessments were made by administering intraperitoneally (IP) either 0, 10, 20, or 40 mg/kg of racemic MDMA HCl and sacrificing by cervical dislocation at 3, 6, or 24 hr posttreatment. To assess the effect of desipramine (DMI) pretreatment on MDMA-induced changes in brain and cardiac norepinephrine levels, either a single dose or three daily doses of 25 mg/kg dose of DMI were administered IP to two groups (n = 6) of mice. Two pretreatment control groups were administered saline. Forty-five minutes following the last dose, one group from each pretreatment condition received 40 mg/kg MDMA and the remaining group received saline. Animals were sacrificed 3 hours following MDMA treatment. At the time of sacrifice, hearts and brains (minus cerebellum) were removed and frozen at −70°C for subsequent analysis of biogenic amines and metabolites by high-performance liquid chromatography with electrochemical detection (HPLC-EC).

Tissue Preparation

Tissues were weighed and homogenized in 1 ml of 0.1 N

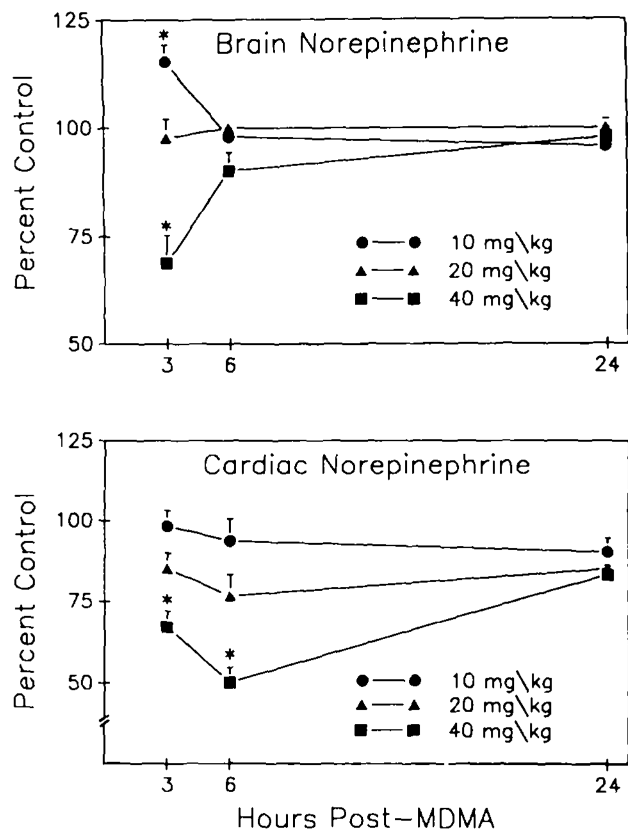

FIG. 3. Effect of acute administration of MDMA on mouse brain and cardiac levels of norepinephrine. Experimental conditions and statistical analyses are described in Fig. 1.

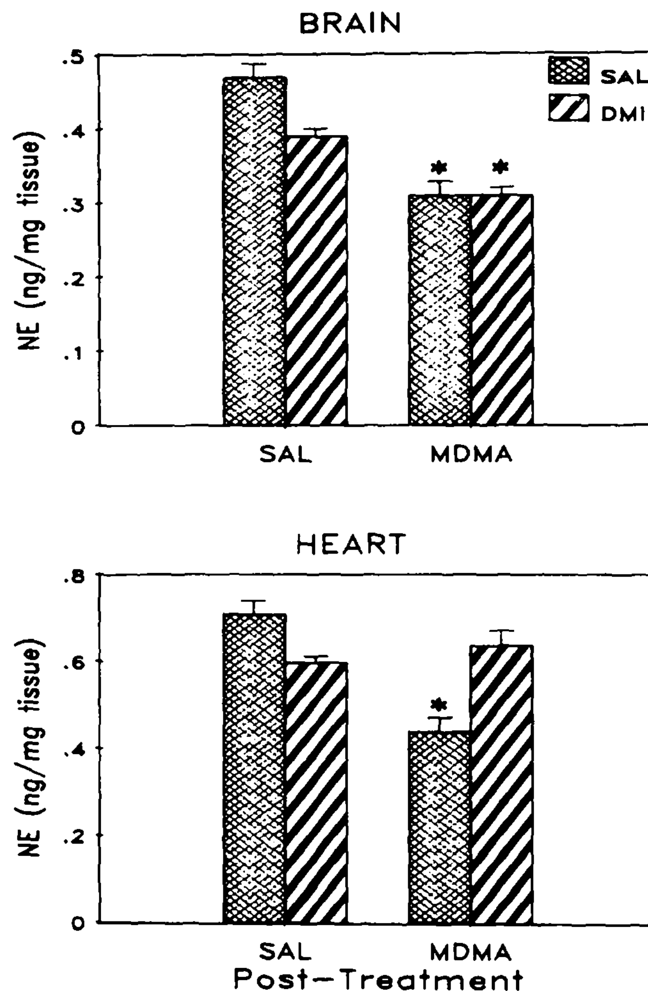

FIG. 4. Effect of desipramine pretreatment on MDMA-induced depletion of brain and cardiac norepinephrine. Male CF-1 mice were randomly assigned to one of four treatment groups (n = 6), two of which were treated IP with desipramine HCl (25 mg/kg) (cross-hatched bars) and the remaining groups were treated with saline (striped bars). One group from each pretreatment condition was administered either 40 mg/kg (±)-MDMA HCl or saline (IP) 45 minutes later. Mice were sacrificed 3 hours post-MDMA. Shown are the means ± S.E.M. for each treatment group. *p<0.05 vs. posttreatment controls (SAL) by a two-way analysis of variance.

perchloric acid containing 0.1% sodium bisulfite, 0.05% EDTA disodium, and 50 ng dihydroxybenzylamine as the internal standard. Samples were centrifuged at 30000 × g for 20 min. The supernatant from the brain extract was injected directly into the LCEC analyzer. Catecholamines from heart tissue were extracted with alumina as described by Eriksson and Persson (10). The supernatant was collected and transferred to a 5 ml reaction vial containing approximately 50 mg alumina. One ml of 0.15 M Tris HCl/0.05 M EDTA (pH 8.6) was added to the vial, which was vortexed rapidly and placed in a reciprocal shaker for 10 min. After shaking, the alumina was washed three times with 1.0 ml aliquots of 3 mM EDTA. The alumina was transferred as a slurry in one ml water to a polypropylene microfuge tube with a filtration assembly (BAS) and was centrifuged for 2 min in a BAS microfuge. Catecholamines were eluted by adding 0.2 ml of the perchloric acid solution to the alumina on the filter and centrifuging. A norepinephrine standard containing 50 ng NE and 50 ng DHBA in one ml phosphate buffer was prepared and handled identically to the supernatant.

HPLC-EC Analysis

Samples were analyzed on an HPLC-EC system consisting of a PM-30A pump, a 25 cm × 4.6 mm (i.d.) Phase II column and an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN). The mobile phase used for analysis of the brain biogenic amines and metabolites contained 75% phosphate/citrate buffer (0.05 M NaH_2PO_4, 0.03 M citric acid, 1.53 mM OSS and 0.1 mM EDTA; pH 2.75) and 25% methanol. Flow rate was 1 ml/min. For analysis of heart extracts, the mobile phase contained 92% phosphate buffer (0.075 M NaH_2PO_4, 0.88 mM OSS and 0.1 mM EDTA; pH 3.0) and 8% acetonitrile pumped at a flow rate of 1.6 ml/min. For all analyses, the applied potential of the detector was 800 mV. Brain samples were quantitated by interpolation of peak height ratios (analyte/DHBA) in samples on a standard curve. NE in heart samples was quantitated as described previously (10).

Statistical Analysis

Data were analyzed using a two-way analysis of variance (dose × time or pretreatment × posttreatment) followed by post hoc Newman-Keuls test when the probability of significant differences in means was less than 0.05.

TABLE 1

WHOLE BRAIN BIOGENIC AMINE AND METABOLITE LEVELS AFTER MDMA IN MICE

Analyte	MDMA Dose (mg/kg) 0	10	20	40
Ne	0.47 ± 0.01	0.47 ± 0.01	0.44 ± 0.01	0.43 ± 0.02
DA	1.41 ± 0.05	1.45 ± 0.02	1.55 ± 0.05	1.25 ± 0.05*
DOPAC	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
HVA	0.50 ± 0.02	0.45 ± 0.04	0.43 ± 0.02	0.38 ± 0.01
5HT	0.72 ± 0.02	0.68 ± 0.03	0.76 ± 0.03	0.73 ± 0.02
5HIAA	0.26 ± 0.01	0.22 ± 0.01*	0.22 ± 0.01*	0.21 ± 0.01*

Mice were sacrificed 1 week after 4 daily IP injections of (+)-MDMA. Results are mean ± S.E.M. ng/mg tissue for 6 animals.
*$p<0.05$ vs. saline-treated controls by one-way ANOVA.

RESULTS

The time course and dose dependence of the effects of acute administration of MDMA on mouse brain 5-HT and 5-HIAA levels are shown in Fig. 1. At the highest dose tested (40 mg/kg), a significant elevation in mouse brain 5-HT was observed 3 hr following drug administration. Levels were not significantly altered from control values with any dose tested at 6 and 24 hr posttreatment. In contrast, mouse brain levels of the 5-HT metabolite, 5-HIAA, were significantly lowered by all three doses of MDMA at 3 hr posttreatment, and at six hours following 40 mg/kg MDMA. Levels of 5-HIAA returned to control values by 24 hr post-MDMA treatment.

The effects of acute administration of (±)-MDMA on mouse brain dopamine and its acidic metabolite DOPAC are depicted in Fig. 2. Brain levels of dopamine were not significantly altered by MDMA, but a significant decline in levels of DOPAC was observed 3 hr posttreatment with the two highest doses. Levels had returned to control values by 24 hr posttreatment. No significant MDMA-induced alterations in mouse brain HVA levels were noted (data not shown).

MDMA induced only transient changes in norepinephrine levels in mouse brain and heart tissue (Fig. 3). Only treatment with the highest dose tested produced significant depletion in either tissue, while the lowest dose (10 mg/kg) caused a significant elevation in brain norepinephrine 3 hours posttreatment. Peak effects occurred in the brain at 3 hr and in the heart at 6 hr following MDMA treatment.

To determine if, as suspected, the MDMA-induced alterations in brain and heart norepinephrine were dependent on carrier-mediated uptake of the drug into presynaptic noradrenergic nerve terminals, the effect of pretreatment with the norepinephrine uptake inhibitor desipramine (DMI) was assessed. Surprisingly, pretreatment of animals with a single dose of DMI 45 minutes prior to administration of 40 mg/kg MDMA only blocked changes in cardiac norepinephrine; brain levels were still significantly depleted by MDMA (Fig. 4). To test the possibility that the DMI regimen was not sufficient to produce the desired central effects, animals were subjected to a repetitive DMI dosing regimen consisting of a single daily dose of DMI for three days, with the last dose administered 45 min prior to 40 mg/kg MDMA. The results obtained were similar to those of the single DMI dose study; only the depleting effects of MDMA on cardiac norepinephrine were blocked (data not shown).

To determine if a subacute treatment regimen of MDMA produced any persistent alterations in mouse brain biogenic amines and metabolites, animals were treated with a single dose of MDMA for four days and sacrificed one week posttreatment. As shown in Table 1, only brain 5-HIAA levels were significantly lowered by all three doses of MDMA. The highest dose tested also produced a significant decrease in brain levels of dopamine and homovanillic acid. Cardiac norepinephrine levels were not significantly altered by this MDMA treatment regimen.

DISCUSSION

The studies presented here, demonstrating a lack of persistent MDMA-induced alterations in mouse brain serotonergic parameters, are consistent with previous studies in which drug effects in mouse and rat (22) and mouse, rat, and guinea pig (1) were directly compared. The observed transient elevation of mouse brain 5-HT contrasts with the early declines that have been reported following MDMA (11) or PCA (19) administration to mice. It is possible that these divergent findings are due to differences in mouse strains. Of greater interest is the striking species variation in response to MDMA between mouse and rat, a species in which drastic declines in brain serotonergic parameters are observed after acute administration of doses similar to those employed here. The unaltered levels of mouse brain 5-HT one week following four consecutive doses of MDMA, a regimen that would produce long-lasting 5-HT depletion in the rat, further exemplifies the resistance of this species to MDMA.

An interesting interpretation of the time course and dose dependence of changes in 5-HT and 5-HIAA following MDMA may provide insight into the basis for the species variation. The transient elevation of 5-HT which is mirrored by a decrease in 5-HIAA suggests that the drug is altering the turnover of 5-HT. Since amphetamines in general have weak MAO inhibitory properties due to the presence of the α-methyl group, it is conceivable that MDMA may have a similar action, thus preventing the degradation of the parent amine and the formation of the metabolite. This raises the question of whether inhibition of MAO in the mouse decreases susceptibility to MDMA neurotoxicity. Interestingly, Mokler *et al.* (13) found that rat brain regions with lower rates of conversion of 5-HT to 5-HIAA following MDMA, which may reflect a decrease in MAO activity, were more resistant to the drug's neurotoxic effects than were regions with higher conversion rates. It is possible that MDMA is an effective MAO inhibitor in the mouse, but not the rat, which protects against drug-induced damage; in the rat, similar protection may be afforded by innately lowered regional MAO activity. The finding that nialamide protects against PCA-induced 5-HT depletion (12) is consistent with this argument. These suggestions imply that active MAO is critical for the neurotoxicity of MDMA to be expressed.

The potent inhibitory effect of MDMA on synaptosomal uptake of [^{3}H]-norepinephrine (18) led to the prediction that the drug would also induce release of norepinephrine, since these two mechanisms are generally affected coincidentally by amphetamine-related compounds. However, acute treatment with MDMA has not been reported to alter norepinephrine levels in rat brain or heart. We report here that a high dose of MDMA produces a transient depletion of brain and cardiac norepinephrine in the mouse. The observation in cardiac tissue is of interest in view of the elevated cardiovascular parameters and related sympathomimetic side effects of the drug reported to occur in humans following ingestion of MDMA (9). Although brain and heart were affected by MDMA, the results of the DMI studies suggest that the depleting effects occur through two different mechanisms. In the heart, carrier-mediated uptake of MDMA appears to be required, but in the brain MDMA is able to produce its depleting effects when the uptake mechanism is presumably shut down. Since the single dose DMI regimen employed has been shown to be effective

at preventing 5,7-dihydroxytryptamine-induced depletion of central norepinephrine stores (3), it is unlikely that insufficient brain levels of DMI account for the lack of protection. The ineffectiveness of the repetitive DMI dosing regimen further argues against this possibility. If the norepinephrine-depleting effects of MDMA in mouse brain are dependent on drug accumulation by the neuron, MDMA must enter the central noradrenergic nerve terminals through either a non-DMI-sensitive carrier mechanism distinct from that in cardiac tissue, or via non-carrier-mediated (i.e., lipophilic) entry. Alternatively, the depletion may be secondary to drug effects on other neurotransmitter systems, which in turn impact upon noradrenergic nerve terminals.

In summary, mice appear to be a uniquely resistant species to the neurotoxic effects of MDMA. In view of the known difference in susceptibility of rats and mice to the serotonergic neurotoxins PCA and fenfluramine, a similar finding with MDMA is not surprising. However, acute administration of MDMA does induce some interesting transient changes in mouse brain and cardiac biogenic amines and metabolites. A detailed examination of how the mechanisms underlying these changes in the mouse compare to those of the rat may provide some insight into the basis of the species variation. Such studies may be useful in elucidating the mechanism of MDMA neurotoxicity.

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REFERENCES

1. Battaglia, G.; Yeh, S. Y.; DeSouza, E. B. MDMA-induced neurotoxicity: Parameters of degeneration and recovery of brain serotonin neurons. Pharmacol. Biochem. Behav. 29:269; 1988.
2. Battaglia, G.; Yeh, S. Y.; O'Hearn, E.; Molliver, M. E.; Kuhar, M. J.; DeSouza, E. B. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H]-paroxetine labelled uptake sites. J. Pharmacol. Exp. Ther. 242:911; 1987.
3. Bjorklund, A.; Baumgarten, H. G.; Rensch, A. 5,7-Dihydroxytryptamine: Improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. J. Neurochem. 24:833; 1975.
4. Boyce, S.; Kelly, E.; Reavill, C.; Jenner, P.; Marsden, C. D. Repeated administration of N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine to rats is not toxic to striatal dopamine neurones. Biochem. Pharmacol. 33:1747; 1984.
5. Braun, U.; Shulgin, A. T.; Braun, G. Centrally active N-substituted analogs of 3,4-methylenedioxyphenyl-isopropylamine (3,4-methylenedioxyamphetamine). J. Pharmacol. Sci. 69:192; 1980.
6. Chiueh, C. C.; Markey, S. P.; Burns, R. S.; Johannessen, J. N.; Pert, A.; Kopin, I. J. Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. Eur. J. Pharmacol. 100:189; 1984.
7. Commins, D. L.; Vosmer, G.; Virus, R. M.; Woolverton, W. L.; Schuster, C. R.; Seiden, L. S. Biochemical and histological evidence that methylenedioxy-methamphetamine (MDMA) is toxic to neurons in rat brain. J. Pharmacol. Exp. Ther. 241:338; 1987.
8. Dowling, G. P.; McDonough, E. T.; Bost, R. O. 'Eve' and 'Ecstasy'. A report of five deaths associated with the use of MDEA and MDMA. JAMA 257:1615; 1987.
9. Downing, J. The psychological and physiological effects of MDMA on normal volunteers. J. Psychoactive Drugs 18:335; 1986.
10. Eriksson, B.-M.; Persson, B.-A. Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection. J. Chromatogr. 228:143; 1982.
11. Fuller, R. W.; Hahn, R. A.; Snoddy, H. D.; Wikel, J. H. Depletion of cardiac norepinephrine in rats and mice by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Biochem. Pharmacol. 33:2957; 1984.
12. Martin, L. L.; Bouchal, R. L.; Smith, D. J. Ketamine inhibits serotonin uptake *in vivo*. Neuropharmacology 21:113; 1982.
13. Mokler, D. J.; Robinson, S. E.; Rosecrans, J. A. (±)-3,4-Methylenedioxymethamphetamine (MDMA) produces long-term reductions in brain 5-hydroxytryptamine in rats. Eur. J. Pharmacol. 138:265–268; 1987.
14. Peroutka, S. J.; Pascoe, N.; Faull, K. F. Monoamine and metabolites in the cerebrospinal fluid of recreational users of 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"). Res. Commun. Subst. Abuse 8:125; 1987.
15. Ricaurte, G. A.; DeLanney, L. E.; Irwin, I.; Langston, J. W. Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of administration. Brain Res. 446:165–168; 1988.
16. Schmidt, C. J. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 240:1; 1987.
17. Schmidt, C. J.; Wu, L.; Lovenberg, W. Methylenedioxymethamphetamine: A potentially neurotoxic amphetamine analog. Eur. J. Pharmacol. 124:175; 1986.
18. Steele, T. D.; Nichols, D. E.; Yim, G. K. W. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]-monoamines into synaptosomes from different regions of rat brain. Biochem. Pharmacol. 36:2297; 1987.
19. Steranka, L. R.; Sanders-Bush, E. Long-term effects of continuous exposure to p-chloroamphetamine on central serotonergic mechanisms in mice. Biochem. Pharmacol. 27:2033; 1978.
20. Steranka, L. R.; Sanders-Bush, E. Species difference in the rate of disappearance of fenfluramine and its effects on brain serotonin neurons. Biochem. Pharmacol. 28:3103; 1979.
21. Stone, D. M.; Stahl, D. C.; Hanson, G. R.; Gibb, J. W. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur. J. Pharmacol. 128:41; 1986.
22. Stone, D. M.; Hanson, G. R.; Gibb, J. W. Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. Neuropharmacology 26:1657; 1987.
23. Stone, D. M.; Merchant, K. M.; Hanson, G. R.; Gibb, J. W. Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26:1677; 1987.