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IN VITRO AND *IN VIVO* NEUROCHEMICAL EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE ON STRIATAL MONOAMINERGIC SYSTEMS IN THE RAT BRAIN

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Abstract—A single high dose of methylenedioxymethamphetamine, a psychedelic agent, produced a rapid and persistent depletion of striatal indoles similar to that observed following administration of the serotonergic neurotoxin *p*-chloroamphetamine. The drug had little effect on dopaminergic variables. Like *p*-choloroamphetamine, methylenedioxymethamphetamine was found to be a relatively selective agent for inducing [^{3}H]serotonin release *in vitro*. The serotonin uptake inhibitor, citalopram, blocked both [^{3}H]serotonin release *in vitro* and striatal serotonin depletion *in vivo*, indicating that both processes were carrier dependent. *In vivo* comparisons of the stereoisomers of methylenedioxymethamphetamine indicated two phases of serotonin depletion similar to those reported for *p*-chloroamphetamine. Although both the (+)- and (−)-stereoisomers produced an acute (3 hr) decrease in striatal indoles, the long-term effects of the drug showed stereoselectivity in that the (+)-enantiomer produced the most dramatic serotonin depletion. Comparison of the effects of the stereoisomers of methylenedioxymethamphetamine and its *n*-desmethyl analog, methylenedioxyamphetamine, on [^{3}H]serotonin and [^{3}H]dopamine release *in vitro* showed the (+)-enantiomer of both drugs to be the more potent releasing agent. In spite of its reported lack of hallucinogenic activity, (+)methylenedioxyamphetamine was found to be of a potency similar to that of (+)methylenedioxymethamphetamine in inducing [^{3}H]serotonin release *in vitro*. The results are discussed in terms of the neurochemical similarities between methylenedioxymethamphetamine and *p*-chloroamphetamine as well as the proposed role of serotonin release in the behavioral effects of methylenedioxymethamphetamine.

(±)Methylenedioxymethamphetamine (MDMA) is a 3,4-methylenedioxy-substituted phenylisopropylamine structurally related to CNS stimulants, such as amphetamine, and to hallucinogens, such as mescaline. Known to abusers as Ecstasy or Adam, MDMA has recently received considerable attention due to its widespread recreational use and subsequent emergency classification as a Schedule I drug by the Food and Drug Administration.

As might be expected from its chemical structure, MDMA elicits behavioral effects characteristic of both the amphetamines and the hallucinogenic phenylalkylamines [1]. However, its effects are described as qualitatively unique in that the user's sense of reality is not distorted but enhanced and there are reportedly no frank hallucinations [1]. The general term psychedelic [2] is therefore a more appropriate label for this agent rather than hallucinogen.

The well-described neurotoxicity of a number of amphetamine derivatives for specific monoaminergic neurotransmitter systems in the rat brain previously led us to examine whether MDMA might cause similar neurochemical alterations. A single high dose of (±)MDMA was found to cause a dramatic depletion of serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in a number of serotonergic terminal regions including the striatum, hippocampus and cerebral cortex [3]. Neostriatal concentrations of 5-HT are still depressed markedly 1 week after a single administration of MDMA. This effect of MDMA is apparently selective for serotonergic neurons since only transient alterations were observed in several neurochemical variables of dopaminergic function. Although the mechanism of this long-term depletion of 5-HT is unknown, the possibility of a neurotoxic basis prompted us to examine further the neurochemical actions of MDMA.

The persistence of MDMA-induced 5-HT depletion is similar to that observed after acute administration of the serotonergic neurotoxin *p*-chloroamphetamine (PCA) or very high doses of methamphetamine (METH) [4, 5]. Both drugs can cause acute and long-term depletions of 5-HT although their long-term effects are probably mediated by different mechanisms [6, 7]. Due to the similarities between MDMA and PCA we further examined the amphetamine-like properties of MDMA. Since a primary neurochemical effect of amphetamines is their carrier-mediated release of monoaminergic neurotransmitters [8], we first studied the effects of MDMA on the release of [^{3}H]dopamine (DA) and [^{3}H]5-HT from superfused rat striatal slices. MDMA-induced transmitter release was characterized and compared to that induced by PCA and METH.

The *n*-desmethyl analog of MDMA, methylenedioxyamphetamine (MDA), has been demonstrated recently to be neurotoxic [9]. While this suggests that a similar mechanism may be responsible for the depletion of 5-HT caused by MDMA, a number of differences between the two agents

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requires any such hypothesis to be tested carefully. For example, an interesting aspect of the pharmacology of the two drugs is that "*R*" (−)MDA is the stereoisomer possessing psychedelic activity whereas in the case of MDMA the "*S*" (+)-stereoisomer is reported to be the behaviorally active enantiomer [10]. Beyond apparent qualitative differences in the intoxication caused by the two drugs [10], this difference in stereochemical requirements indicates that the behavioral effects of MDMA are not due to its metabolism to MDA. This is also supported by the reported lack of cross-tolerance between the two drugs [10]. Since some of the behavioral effects of several psychedelic amphetamine derivatives have been ascribed to their effects on neurotransmitter release, particularly 5-HT [11, 12], it was of interest to compare the stereoisomers of MDA and MDMA for their effects on [^{3}H]DA and [^{3}H]5-HT release *in vitro*. The stereochemical requirement for transmitter release was also compared to the ability of the stereoisomers of MDMA to induce both the acute and long-term neurochemical alterations previously reported for the racemic drug.

METHODS

Release studies. All release experiments were conducted using superfused rat striatal slices preloaded with either [^{3}H]DA or [^{3}H]5-HT. For each experiment two male Sprague–Dawley rats (200–500 g) were decapitated, their brains were removed immediately, and both neostriata were dissected free on ice. Striatal slices (0.25 × 0.25 mm) were prepared using a McIlwain Tissue Chopper. Slices were incubated for 10 min at 37° in 5 ml of Krebs–Ringer bicarbonate buffer (KRB) containing (mM): NaCl (118), KCl (4.85), CaCl (2.5), $MgSO_4$ (1.15), KH_2PO_4 (1.15), $NaHCO_3$ (25), and glucose (11.1) at pH 7.3. All solutions also contained 10^{-4} M pargyline and were bubbled constantly with 95% O_2–5% CO_2. [^{3}H]DA (New England Nuclear, Boston, MA, 7.5 Ci/mmole) or [^{3}H]5-HT (New England Nuclear, 8 Ci/mmole) was added at 10^{-7} M, and the incubation was continued for an additional 15 min. After washing, the slices were aliquoted between eight superfusion chambers [13] set to a volume of approximately 0.25 ml. The chambers were then superfused with KRB at 0.5 ml/min for 30 min to achieve a stable baseline efflux of radioactivity. During the determination of drug-induced release, effluent from the chambers was collected in 5-min fractions directly into liquid scintillation vials for counting. At the termination of each experiment the slices were removed from each chamber, solubilized in Protosol (New England Nuclear), and counted in ACS scintillation fluid (Amersham, Arlington Heights, IL) to determine residual radioactivity.

Release in each 5-min period was calculated as a fraction of the total radioactivity present in the slices during that period. To determine drug-induced release, the spontaneous efflux prior to exposure to the drug was subtracted for each chamber.

Alterations of MDMA-induced release was determined using S_2/S_1 ratios. After an initial 5-min exposure to MDMA (S_1) and 15 min prior to the second pulse of MDMA (S_2), half of the chambers were switched to KRB containing the agent of interest. The S_2/S_1 ratios for chambers exposed to the blocking agent during S_2 were then compared to those for chambers superfused with only KRB.

Uptake experiments. Synaptosomal uptake experiments were performed essentially as described by Wagner *et al.* [14]. Briefly, a P_2 pellet was prepared from striata pooled from four rats. The pellet was resuspended in 10 ml KRB, and a 0.2-ml aliquot of this suspension was added to 3.8 ml of KRB containing either [^{3}H]MDMA (1.7 Ci/mmole, 10^{-7} M) or [^{3}H]DA (7.5 Ci/mmole, 10^{-7} M). Total uptake was determined by incubation for 10 min at 37°. Tubes for nonspecific uptake remained on ice at 4° during the incubation. Samples were then filtered on Whatman GF/B filters using a Brandel Cell Harvester (Gaithersburg, MD). The filters were washed twice with 5 ml of cold KRB and placed in scintillation vials. ACS scintillation fluid (Amersham) was added, and the samples were counted to determine uptake. [^{3}H]MDMA (29 Ci/mmole) was synthesized as described below using [^{3}H]methylamine (Amersham, 29 Ci/mmole). The product was isolated by thin-layer chromatography and autoradiography.

In vivo *experiments.* Male Sprague–Dawley rats (200–250 g) were used for all experiments. Animals were maintained on a 12-hr light–dark cycle and given free access to food and water. Drug administration was confined to 8:00 a.m. to 12:00 a.m. due to an increased incidence of mortality at later times. All drugs were administered by subcutaneous injection using saline as the vehicle. At the appropriate times, the animals were decapitated and the striata were removed as already described. Tissues were frozen at −80° until assayed.

Striatal concentrations of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-HIAA were determined by high performance liquid chromatography with electrochemical detection. Briefly, one striata from each animal was weighed and homogenized in 1 ml of mobile phase containing monochloroacetic acid (0.15 M), EDTA (0.2 mM), 1 g/l heptyl sulfonic acid and 5% methanol at pH 2.9. After centrifuging (30,000 *g*, 15 min) the samples were injected onto a Waters 5 μm Nova-a-pak C-18 column. Detection was by means of a Coulochem model 5100A detector (ESA, Inc., Bedford, MA) using a potential of +0.4 V and a guard cell potential of +0.05 V. Monoamines and their metabolites were quantitated by comparison with standards of known concentration using a SP 4270 integrator (Spectra Physics, San Jose, CA.

Drugs. (±)*N*-Methyl-3,4-methylenedioxyphenylisopropylamine hydrochloride (MDMA) was prepared as described by Braun *et al.* [15]. The structure and purity of the product were verified by NMR and elemental analysis respectively. The stereoisomers of MDA and MDMA were provided by the National Institute on Drug Abuse. (±)Methamphetamine and (±)*p*-chloroamphetamine were purchased from the Sigma Chemical Co. (St. Louis, MO). Citalopram hydrobromide and amfonelic acid were gifts of H. Lundbeck & Co. and Sterling-Winthrop respectively.

Table 1. Effect of the acute administration of racemic MDMA on striatal monoamine and metabolite concentrations at 3 hr.

	Monoamine and metabolite concentrations (μg/g tissue)				
	5-HT	5-HIAA	DA	DOPAC	HVA
Control (saline)	0.519 ± 0.036 (100 ± 6.9)	0.390 ± 0.026 (100 ± 6.7)	10.52 ± 0.61 (100 ± 5.8)	1.07 ± 0.03 (100 ± 2.9)	0.654 ± 0.092 (100 ± 14.1)
10 mg/kg (±)MDMA	0.284 ± 0.021* (54.7 ± 4.1)	0.249 ± 0.011† (63.9 ± 2.8)	10.89 ± 0.42 (103.5 ± 4.0)	0.777 ± 0.042* (72.6 ± 3.9)	0.749 ± 0.045 (114.5 ± 6.9)
20 mg/kg (±)MDMA	0.326 ± 0.021† (62.8 ± 4.1)	0.247 ± 0.010† (63.3 ± 2.6)	12.28 ± 0.51 (116.7 ± 4.9)	0.600 ± 0.025* (56.1 ± 2.3)	0.672 ± 0.028 (102.8 ± 4.3)

Results are the mean of five animals ± SEM.
*,† Significantly different from control by the two-tailed Student's *t*-test: * $P < 0.001$, and † $P < 0.01$.

Statistics. Significant differences were determined using the two-tailed Student's *t*-test with a P value of less than 0.05 being accepted as a statistical difference.

RESULTS

Acute effects of MDMA on striatal monoamines. The administration of a single dose of MDMA at either 10 or 20 mg/kg s.c. caused a dramatic decrease in striatal concentrations of both 5-HT and 5-HIAA by 3 hr (Table 1). The degree of this acute depletion was apparently maximal at the 10 mg/kg dose as no further decrease was observed with 20 mg/kg at this single time point.

In contrast to the effect of MDMA on serotonergic variables, the striatal dopaminergic system was much less affected by MDMA. DA itself showed a tendency to increase in concentration although this did not reach statistical significance in this experiment. DOPAC concentrations declined in a dose-dependent manner following MDMA while no significant changes were observed for HVA, the other major metabolite of DA.

Comparison of [^{3}H]*monoamine release from superfused striatal slices by racemic METH, PCA and MDMA*. As already stated, the acute depletion of striatal indoles by MDMA suggests some neurochemical similarities with other amphetamine analogues. Figure 1A compares the *in vitro* release of [^{3}H]DA from striatal slices by METH, PCA and MDMA. Release by all three drugs was concentration dependent over the range of 10^{-6} to 10^{-4} M. METH was the most potent DA-releasing agent of the three, being approximately an order of magnitude more potent than MDMA.

Release of [^{3}H]5-HT from striatal slices by the three drugs is compared in Fig. 1B. In this case MDMA and PCA were considerably more potent releasers of 5-HT than METH. At 10^{-6} M drug, MDMA was approximately an order of magnitude more potent than METH for inducing transmitter release. By comparing panels A and B of Fig. 1, it is also apparent that MDMA and PCA released 5-HT at 10-fold lower concentrations than those required to initiate DA release.

Effect of uptake inhibitors on in vitro *transmitter release by MDMA*. The release of monoaminergic neurotransmitters by amphetamines is generally believed to be dependent upon a functional monoamine uptake system [8]. To determine if the *in vitro* release induced by MDMA occurred through such a mechanism, experiments were performed examining the effect of the DA uptake inhibitor, amfonelic acid, and the 5-HT uptake inhibitor, citalopram, on the MDMA-induced release of [^{3}H]DA and [^{3}H]5-HT respectively.

Figure 2 illustrates the ratio for the amount [^{3}H]DA released in two sequential 5-min pulses (S_1 and S_2) of MDMA (10^{-5} M). Control ratios were determined in the presence of MDMA alone, whereas amfonelic acid (10^{-7} M) was present during S_2 to determine the effect of uptake carrier inhibition. The right side of Fig. 2 provides the results from identical experiments for the effect of citalopram (10^{-7} M) on MDMA-induced [^{3}H]5-HT release. The data from both sets of experiments demonstrate a highly significant inhibition of MDMA-induced transmitter release when the carrier-dependent transport system of the specific monoamine was inhibited.

Effect of citalopram on MDMA-induced 5-HT depletion in vivo. The ability of citalopram to block [^{3}H]5-HT release by MDMA *in vitro* led us to determine the effects of the drug combination *in vivo*. Figure 3 shows the effect of the acute administration of MDMA (10 mg/kg, s.c.) on striatal concentrations of 5-HT and 5-HIAA. MDMA reduced striatal 5-HT and 5-HIAA to 47 and 62% of control, respectively, 3 hr after drug administration. Citalopram (2 mg/kg, s.c.) alone reduced stiratal 5-HIAA concentrations, indicating significant inhibition of 5-HT uptake, although the drug had no effect on 5-HT concentrations. When administered simultaneously with MDMA, however, citalopram completely antagonized the acute depletion of 5-HT produced by MDMA.

As described by Fuller [16], the effect of an uptake inhibitor on amine depletion by an amphetamine may occur by either of two mechanisms. Carrier-dependent transport of the depleting agent may be blocked or carrier-mediated transport of the amine out of the neuron may be inhibited. To determine if

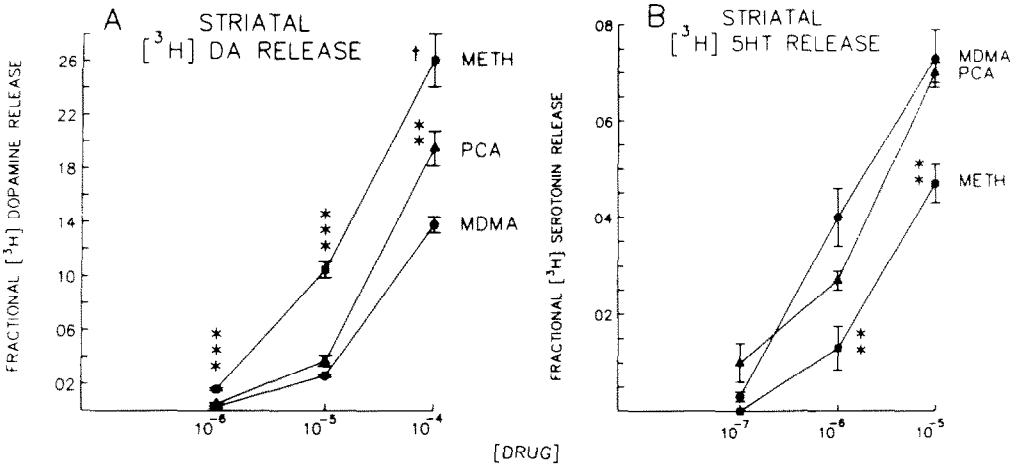

Fig. 1. Release of tritiated monoamines from superfused striatal slices by METH, PCA and MDMA. Release is expressed as a fraction of total tissue stores of radioactivity with drug-induced release being the difference between tritium efflux following a 5-min pulse of drug and basal efflux. Values are the mean ± SEM for an average of eight determinations. Key: (**) $P < 0.1$ vs MDMA, (***) $P < 0.001$ vs MDMA, and (†) $P < 0.001$ vs MDMA and $P < 0.02$ vs PCA.

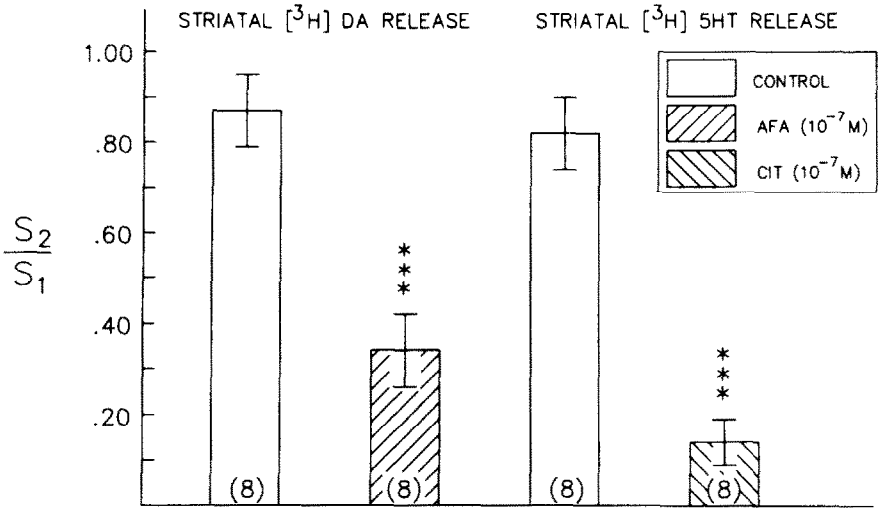

Fig. 2. Effect of selective monoamine uptake inhibitors on the MDMA-induced release of [^{3}H]DA and [^{3}H]5-HT. S_2/S_1 ratios were calculated from the release of tritium induced by two consecutive 5-min pulses of MDMA with S_2 being in the absence (control) or presence of the inhibitor. Key: (***) $P < 0.001$ vs control.

there was significant uptake of MDMA into brain nerve terminals we compared the accumulation of [^{3}H]DA and [^{3}H]MDMA in a preparation of striatal synaptosomes (P_2). Total uptake was determined for 10 min at 37° whereas nonspecific activity was determined at 4°. As shown in Table 2 whereas [^{3}H]DA was avidly accumulated with approximately 94% specific uptake, relatively little specific [^{3}H]MDMA accumulation (18%) could be demonstrated.

Comparison of [^{3}H]*monoamine release* in vitro *by stereoisomers of MDA and MDMA.* The possibility that the stereochemical requirements for the behavioral effects of MDA and MDMA *in vivo* may be reflected in stereospecific effects on neurotransmitter release was evaluated. Figure 4A shows the concentration-dependent release of [^{3}H]DA from superfused rat striatal slices by the stereoisomers of both MDA and MDMA. The "*S*" (+)-stereoisomer of both drugs was found to be a significantly more potent DA releasing agent than the "*R*" (−)-isomer. The identical stereoisomers of each drug were also very similar in potency.

Identical results were observed for [^{3}H]5-HT release by stereoisomers of the two drugs (Fig. 4B) in that the "*S*" (+)-isomers were again the most potent at inducing release. Also, at 10^{-5} M, both (+)MDA and (+)MDMA appeared very similar in potency for increasing [^{3}H]5-HT release. As observed for racemic MDMA (Fig. 1), significant

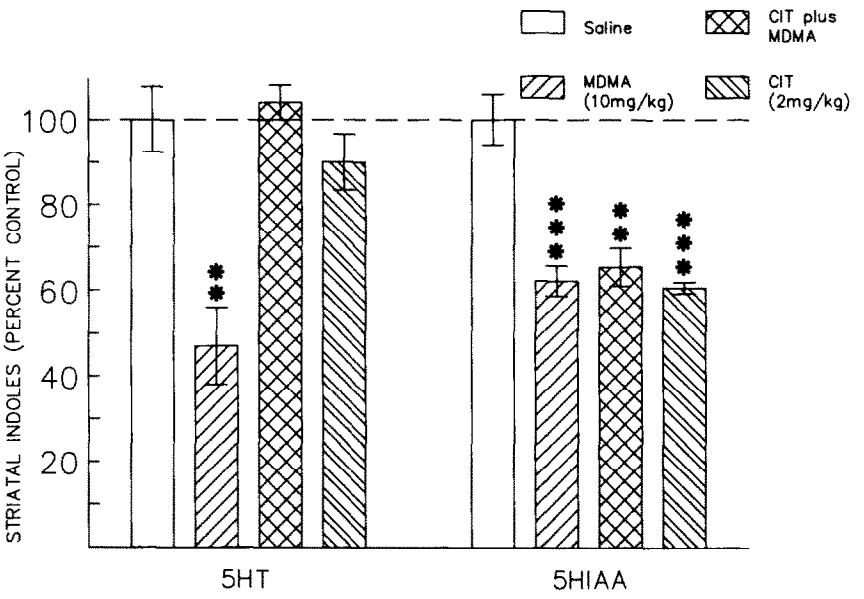

Fig. 3. Effect of the 5-HT uptake inhibitor, citalopram, on the acute depletion of striatal indoles. Drugs were administered simultaneously 3 hr prior to killing the animals. Values are the mean ± SEM for five animals. Key: (**) $P < 0.01$ vs control, and (***) $P < 0.001$ vs control.

Table 2. Uptake of [^{3}H]DA or [^{3}H]MDMA (100 nM) by rat striatal synaptosomes

	[^{3}H]DA (pmoles/mg protein)	[^{3}H]MDMA (pmoles/mg protein)
Total	86.7 ± 1.1	1.22 ± 0.04
Non-specific	5.2 ± 0.1	1.00 ± 0.02
Specific uptake	81.5 (94%)	0.22 (18%)

Total uptake was measured in a P_2 synaptosomal preparation in a 10-min incubation at 37°. Nonspecific uptake was determined at 4°. Each value is the mean of four determinations ± SEM.

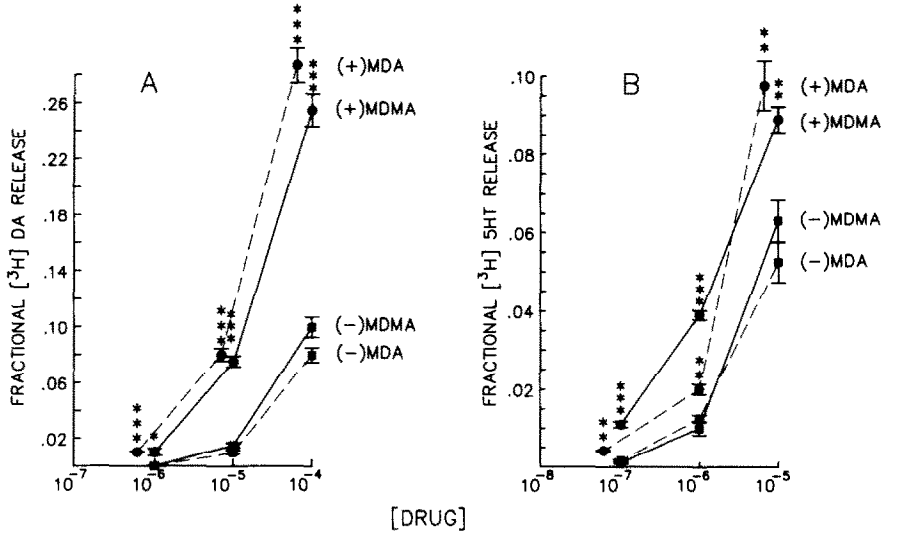

Fig. 4. Release of [^{3}H]DA (A) and [^{3}H]5-HT (B) from superfused striatal slices by the stereoisomers of MDA and MDMA. Results are calculated as described for Fig. 1. Key: (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ vs (−)-stereoisomer.

Table 3. Acute changes (3 hr) in striatal monoamine and metabolite concentrations following administration of (−)- and (+)MDMA

	Monoamine and metabolite concentrations (μg/g tissue)				
	5-HT	5-HIAA	DA	DOPAC	HVA
Control (saline)	0.519 ± 0.036 (100 ± 6.9)	0.390 ± 0.026 (100 ± 6.7)	10.52 ± 0.61 (100 ± 5.8)	1.070 ± 0.031 (100 ± 2.9)	0.654 ± 0.092 (100 ± 14.1)
10 mg/kg (−)MDMA	0.271 ± 0.017* (52.2 ± 3.3)	0.281 ± 0.017† (72.1 ± 4.4)	9.6 ± 4.6 (91.3 ± 4.4)	0.718 ± 0.036* (67.1 ± 3.4)	0.542 ± 0.026 (82.9 ± 4.0)
20 mg/kg (−)MDMA	0.237 ± 0.027* (45.7 ± 5.2)	0.273 ± 0.016† (70.0 ± 4.1)	9.75 ± 0.42 (92.7 ± 4.0)	0.559 ± 0.021* (52.2 ± 2.0)	0.581 ± 0.021 (88.8 ± 3.2)
10 mg/kg (+)MDMA	0.361 ± 0.023†,‡ (69.6 ± 4.4)	0.272 ± 0.006† (69.7 ± 1.5)	12.67 ± 0.56§ (120.4 ± 5.3)	0.620 ± 0.049* (57.9 ± 4.6)	0.592 ± 0.048 (90.5 ± 7.3)
20 mg/kg (+)MDMA	0.402 ± 0.007§,‖ (77.5 ± 1.4)	0.263 ± 0.008† (67.4 ± 2.1)	11.13 ± 0.66 (105.8 ± 6.3)	0.401 ± 0.027*,¶ (37.5 ± 2.5)	0.526 ± 0.047 (80.4 ± 7.2)

Results are the mean of five animals ± SEM.
*,† Significantly different from saline: * $P < 0.001$ and † $P < 0.01$.
‡ Significantly different from (−)MDMA: $P < 0.05$.
§ Significantly different from saline: $P < 0.05$.
‖,¶ Significantly different from (−)MDMA: ‖ $P < 0.001$, and ¶ $P < 0.01$.

release of [^{3}H]5-HT was achieved at 10^{-7} M (+)MDMA, whereas 10-fold higher concentrations were required for [^{3}H]DA release.

Stereochemical requirements for the acute effects of MDMA on striatal monoamine concentrations. In an attempt to correlate the *in vitro* releasing action of MDMA with its *in vivo* effects on neurochemical variables of the serotonergic system, the two stereoisomers of MDMA were administered to rats. The effects of (−)- and (+)MDMA (10 or 20 mg/kg, s.c.) on striatal monoamine concentrations 3 hr after drug administration are shown in Table 3.

Both (−)- and (+)MDMA produced significant reductions in striatal concentrations of 5-HT and 5-HIAA. Both stereoisomers also produced their maximum effect at 10 mg/kg with no further decrement in 5-HT or 5-HIAA concentrations being observed at the 20 mg/kg dose. However, the (−)-isomer caused a significantly greater depletion of striatal 5-HT at both doses when compared to the effect of (+)MDMA. The decreases in 5-HIAA concentrations were similar to both stereoisomers.

In contrast to the dramatic effects of both stereoisomers on striatal serotonergic variables, the effects of MDMA on the dopaminergic system were more apparent with the (+)-stereoisomer. The tendency to elevate striatal DA was not observed with (−)MDMA, whereas (+)MDMA caused a significant elevation of DA concentrations at the 10 mg/kg dose. At 20 mg/kg, (+)MDMA also reduced striatal DOPAC concentrations to a significantly greater extent than did (−)MDMA although both isomers had significant effects on this variable.

Stereochemical requirements for the long-term effects of MDMA on striatal monoamine concentrations. The mechanism responsible for the persistent decrease in 5-HT and 5-HIAA concentrations may be different than that causing the acute depletion of striatal indoles after MDMA. We therefore compared the ability of the stereoisomers of MDMA to produce the persistent depletion in striatal 5-HT and 5-HIAA concentrations previously reported for the racemic drug [3]. Rats were again administered either (−)- or (+)MDMA (10 or 20 mg/kg, s.c.) as in the acute experiments and allowed to survive 7 days until killed. Striatal monoamine concentrations from these animals are provided in Table 4.

In contrast to the acute comparison of the MDMA stereoisomers, at 7 days the effect of (+)MDMA on striatal indole concentrations was significantly greater than that of the (−)-stereoisomer. At 10 mg/kg, the effects of both stereoisomers were reversed at 1 week. However, 5-HT concentrations were still reduced to 58% of control in the 20 mg/kg (+)MDMA group, whereas 5-HT concentrations had returned to 88% of control in the (−)MDMA group at the same dose. Striatal 5-HIAA concentrations were also reduced significantly in this (+)MDMA group (72% of control).

The only significant effects observed in the dopaminergic variables were in the 20 mg/kg (+)MDMA group. These animals all showed a significant reduction in striatal DA and DOPAC concentrations to approximately 74% of control. A similarly depressed value for striatal HVA did not reach statistical significance.

DISCUSSION

The results of the present study demonstrate a number of neurochemical similarities between MDMA and the structurally related amphetamines. For example, MDMA is a potent releaser of both [^{3}H]DA and [^{3}H]5-HT *in vitro* by a carrier-dependent mechanism as demonstrated by the effect of selective inhibitors of monoamine uptake. Furthermore, release of both transmitters by MDMA was not inhibited by removal of Ca^{2+} from the superfusion media (Schmidt, unreported observation). Similar results have been demonstrated for a number

Table 4. Long-term changes in striatal monoamine and metabolite concentrations one week following administration of (−)- or (+)MDMA

	Monamine and metabolite concentrations (μg/g tissue)				
	5-HT	5-HIAA	DA	DOPAC	HVA
Control (saline)	0.566 ± 0.008 (100 ± 1.4)	0.384 ± 0.015 (100 ± 3.9)	9.22 ± 0.21 (100 ± 2.3)	0.957 ± 0.069 (100 ± 7.2)	0.574 ± 0.059 (100 ± 10.3)
10 mg/kg (−)MDMA	0.500 ± 0.041 (88.3 ± 7.2)	0.340 ± 0.026 (88.5 ± 6.8)	9.32 ± 0.45 (101.1 ± 4.9)	0.965 ± 0.049 (100.8 ± 5.1)	0.643 ± 0.057 (112.0 ± 9.9)
20 mg/kg (−)MDMA	0.499 ± 0.015* (88.2 ± 2.7)	0.349 ± 0.008 (90.9 ± 2.1)	9.03 ± 0.26 (97.9 ± 2.8)	0.919 ± 0.035 (96.0 ± 3.7)	0.566 ± 0.044 (96.6 ± 7.7)
10 mg/kg (+)MDMA	0.472 ± 0.051 (83.4 ± 9.0)	0.358 ± 0.034 (93.2 ± 8.9)	9.54 ± 0.49 (103.5 ± 5.3)	0.954 ± 0.041 (99.7 ± 4.3)	0.606 ± 0.039 (105.6 ± 6.8)
20 mg/kg (+)MDMA	0.327 ± 0.029†,‡ (57.8 ± 5.1)	0.276 ± 0.024*,§ (71.9 ± 6.3)	6.89 ± 0.36† (74.7 ± 3.9)	0.708 ± 0.026* (74.0 ± 2.7)	0.437 ± 0.029 (76.1 ± 5.1)

Results are the mean of five animals ± SEM.
*,† Significantly different from saline: * $P < 0.01$, and † $P < 0.001$.
†,§ Significantly different from (−)MDMA: ‡ $P < 0.001$, and § $P < 0.05$.

of amphetamine analogues [8, 13, 17, 18]. The greater potency of the (+)-stereoisomer of MDMA compared to the (−)-stereoisomer as a releasing agent has also been demonstrated for amphetamine [19].

The ability of uptake inhibitors to interfere with the MDMA-induced release of monoamines can be explained by one of two mechanisms. If such release occurs through an exchange-diffusion mechanism as described by Fisher and Cho [20] and others [21], uptake inhibitors could block the carrier-mediated transport of MDMA into the nerve terminal. This is an attractive option in view of the ability of citalopram to antagonize both MDMA-induced [^{3}H]5-HT release *in vitro* and the acute depletion of striatal 5-HT produced by MDMA administration *in vivo*. Alternatively, the inhibitor may prevent the carrier-mediated transport of monoamines out of the nerve terminal while having no effect on the entrance of the releasing agent (i.e. MDMA) into the neuron [13, 22, 23]. This latter explanation seems the most plausible in the case of relatively lipophilic drugs such as MDMA. Furthermore, we did not find evidence for an active accumulation of [^{3}H]MDMA by rat striatal synaptosomes, suggesting, that MDMA probably enters the neuron by passive mechanisms where it causes the displacement and release of monoamines. Regardless of the mechanism, the antagonism of the *in vitro* and the acute *in vivo* effects of MDMA by an uptake inhibitor was identical to that reported for the serotonergic neurotoxin, PCA [24, 25]. The results demonstrate a role for the uptake carrier in the acute MDMA-induced depletion of 5-HT.

MDMA also appears similar to PCA in terms of its more selective effects on [^{3}H]5-HT release as compared to [^{3}H]DA release from striatal slices. It is worth mentioning that no stereotypy was observed in any of our *in vivo* experiments with MDMA except at the highest doses of the (+)-stereoisomer. In our release system, MDMA appeared more closely related to PCA than METH, a drug that might be considered structurally more similar to MDMA. The basis of the greater selectivity of MDMA for inducing 5-HT release is unknown but is interesting in view of the behavioral effects of the drug and the generally held view that the serotonergic system plays an important role in the actions of psychedelic agents [26–32].

In addition to the similarities between PCA and MDMA as releasing agents *in vitro*, the two drugs share a number of neurochemical effects *in vivo*. Both PCA and MDMA produce acute and long-term depletions of striatal 5-HT concentrations after a single administration [24, 25, 33]. The acute depletion of 5-HT concentrations by PCA is believed to be due to an inhibition of both 5-HT synthesis and reuptake coupled with an increase in transmitter release [4, 16, 33]. In contrast to its acute effects, the long-term depletion of 5-HT produced by PCA has been associated with a neurotoxic response and the degeneration of serotonergic nerve terminals and cell bodies [34, 35]. Inhibition of MDMA-induced 5-HT release by citalopram *in vitro* and blockade of MDMA-induced 5-HT depletion by citalopram *in vivo* suggest that at least the acute effects of MDMA and PCA are due to a similar mechanism, i.e. a marked increase in utilization of the transmitter with no compensating increase in synthesis. Inhibition of monoamine oxidase by MDMA, as indicated by the acute reduction in the concentration of DOPAC (Table 1), would, of course, block the increase in 5-HIAA concentrations which should normally accompany an increase in 5-HT release. It should be noted that the failure of MDMA to modify HVA concentrations has also been observed following amphetamine administration in spite of its documented ability to lower DOPAC concentrations through inhibition of MAO. This has been suggested to be due to amphetamine's preferential inhibition of MAO_A which is intraneuronal in DA neurons and thus responsible for the formation of DOPAC.

MAO_B, which is primarily extraneuronal, would still be available for the conversion of released DA to HVA [36]. A similar explanation probably accounts for the different effects of MDMA on DOPAC and HVA concentrations in this study.

METH is a closely related drug which has also been shown to produce both an acute depletion of 5-HT and a neurotoxic response in serotonergic neurons when administered at high doses to laboratory animals [5, 37]. Unlike PCA, the 5-HT depletion and neurotoxicity of METH have been linked to its ability to release DA [7]. As demonstrated in this study, METH is at least an order of magnitude more potent at releasing DA than is MDMA. Taken with the fact that MDMA and METH acutely deplete 5-HT to a similar degree [18], this observation suggests that an increase in DA release may not be involved in the acute depletion of 5-HT following MDMA administration. In the case of PCA-induced 5-HT depletion, the long-term effects are suggested to be due to the formation of a chemically reactive metabolite which is selectively toxic to serotonergic neurons [6, 38]. A similar mechanism (i.e. a toxic metabolite) may be responsible for the long-term 5-HT depletion produced by MDMA. The stereospecificity of the long-term effect of MDMA on striatal 5-HT concentrations reported here supports this suggestion since similar stereoselectivity has been observed for the neurotoxic effects of PCA. Both (+)- and (−)PCA have been shown to produce an acute depletion of whole brain 5-HT in rats; however, the loss of neurotransmitter concentrations observed at 2 weeks is greater for the (+)-stereoisomer [39]. This stereoselective effect is not due to differences in the metabolism or disposition of the two enantiomers of PCA since brain concentrations of both stereoisomers are similar and decline at identical rates following drug administration [39]. Unfortunately, little information is currently available on the metabolism of MDMA; hence, differences in disposition of the enantiomers cannot be eliminated as an explanation for the stereoselectivity of the long-term effect of MDMA. However, the similarity of this long-term effect for the (+)-stereoisomers of both PCA and MDMA is in accord with a common mechanism and may suggest that the neurotoxicity recently reported for MDA [9] is also a property of the (+)-stereoisomer and, therefore, not due to the hallucinogenically active form of the drug. This remains to be tested, however.

It has been suggested that hallucinogenic phenylalkylamines form a continuum from those having only direct receptor effects to those having effects primarily through their ability to cause the release of endogenous neurotransmitters [11, 12]. Methoxylated amphetamines such as *p*-methoxyamphetamine (PMA) often have potent amphetamine-like stimulant effects, indicating significant releasing action [12]. Like MDMA and PCA, PMA is reported to be a poor releaser of DA in comparison to agents such as amphetamine [40]; however, it is much more potent than amphetamine at releasing 5-HT [41]. Such findings may explain the absence of stereotypy following PMA [42] or MDMA (this study) administration. PMA-induced 5-HT release has been suggested to be the basis of its hallucinogenic effects since PMA reportedly has little or no direct action at 5-HT receptors [26, 43, 44]. A similar hypothesis has been presented for the psychedelic effects of MDA and MDMA [43].

However, experiments examining the abilities of the stereoisomers of MDA and MDMA to induce 5-HT release are inconsistent with this view. As already described, the "*R*" (−)-stereoisomer of virtually all phenylalkylamines is the active psychedelic agent behaviorally [10, 32, 43, 45, 46]. A second general rule seems to be that the N-alkylation of such compounds causes a decrease in their potency [10, 12, 15, 43]. MDMA constitutes an exception to both rules. Although it is the "*R*" stereoisomer of MDA which is the active hallucinogen in humans, N-methylation of this compound to MDMA produces a unique agent of similar psychedelic potency which is reportedly active in the "*S*" (+) configuration [10, 12, 15, 43]. This reversed stereoselectivity of MDMA and the observation that the (+)-stereoisomer is more potent than (−)MDMA at releasing [^{3}H]5-HT from rat brain synaptosomes led to the suggestion that 5-HT release may be responsible for its psychedelic effects. This would be in contrast to related phenylalkylamines in which direct receptor activation is believed to play a primary role in their behavioral effects. Although we have observed a similar stereoselectivity for the MDMA-induced release of [^{3}H]5-HT in the present study, it seems unlikely that most of the psychedelic effects of MDMA could be due to 5-HT release. If this were the case, our *in vitro* data would suggest that (+)MDA should be a psychedelic agent similar to (+)MDMA since their effects on [^{3}H]5-HT release were comparable. However, drug discrimination studies have demonstrated that (+)MDA is behaviorally a CNS stimulant which generalizes to an amphetamine stimulus but does not generalize to a hallucinogenic agent like 1-(2,5-dimethody-4-methylphenyl)-2-aminopropane (DOM). The effects of (−)MDA, however, are similar to DOM or LSD in the same test [47, 48]. This suggests that neurochemical events more complex than 5-HT release are involved in the psychedelic effects of MDMA and possibly related agents. Recently we presented evidence for MDMA binding site in the rat brain which is apparently distinct from the 5-HT receptor [49]. Studies are currently in progress to further characterize this binding site and to determine if it may, in fact, mediate some of the unique central effects of MDMA.

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