Schmidt 1987 105

0022-3565/87/2401-0001$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 240, No. 1
Printed in U.S.A.

Neurotoxicity of the Psychedelic Amphetamine, Methylenedioxymethamphetamine

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Accepted for publication September 15, 1986

ABSTRACT

The neurochemical effects of the unique psychedelic agent, methylenedioxymethamphetamine (MDMA), indicate it may be a serotonergic neurotoxin related to agents such as *p*-chloroamphetamine. MDMA had a biphasic effect on cortical serotonin concentrations beginning with an acute depletion of the transmitter which reached a maximum between 3 and 6 hr after drug administration. This early phase of depletion was reversible because cortical serotonin concentrations had recovered to control levels by 24 hr. However, transmitter concentrations were reduced significantly 1 week later, indicating a second phase of depletion. The latter phase of depletion was associated with a decrease in synaptosomal [^{3}H]serotonin uptake due to a loss in the number of uptake sites with no change in the affinity of the carrier for serotonin. This neurotoxic effect of MDMA was found to be a property of the (+)-stereoisomer of the drug as only this enantiomer produced the depletion of cortical serotonin and the decrease in synaptosomal serotonin uptake at 1 week. In contrast to this, both stereoisomers of the drug could produce the acute depletion of cortical serotonin measured 3 hr after drug administration. Coadministration of the selective serotonin uptake inhibitor, fluoxetine, completely blocked the reduction in cortical serotonin concentrations 1 week after MDMA. Administration of fluoxetine at various times after MDMA revealed that the long-term effects of the drug developed independently of the acute depletion of serotonin and could be partially blocked by the uptake inhibitor as long as 6 hr after drug administration. The results demonstrate a neurotoxic effect of MDMA on serotonergic nerve terminals and suggest a neurotoxic intermediate may be responsible.

3,4-MDMA is a novel psychedelic amphetamine analog classified recently as a Schedule I compound due to its widespread abuse and suggestions that the drug may be neurotoxic. The latter concern rose out of the observation that the N-desmethyl parent of MDMA, 3,4-MDA, caused the selective degeneration of serotonergic nerve terminals in rats administered high doses of the drug (Ricaurte *et al.*, 1985). This effect is accompanied by a decrease in the concentration of 5-HT in a number of brain regions as well as a decrease in the activity of tryptophan hydroxylase, the rate-limiting enzyme for 5-HT synthesis (Stone *et al.*, 1986).

We have reported recently that MDMA itself may also be neurotoxic in rats at acute parenteral doses approximately four times the acute human oral dose (Schmidt *et al.*, 1986a). Our results demonstrated a significant and selective depletion of 5-HT and its metabolite, 5-hydroxyindoleacetic acid, by MDMA in several brain areas. In the striatum, this depletion was still significant 1 week after the administration of MDMA at doses of 10 mg/kg or greater. In contrast, MDMA had no significant long-term effects on dopaminergic parameters in the same experiments.

Received for publication July 7, 1986.

Although a number of amphetamine analogs have been found to be neurotoxic at high doses (Fuller, 1985), the neurochemical effects of MDMA most closely resemble those of the serotonergic neurotoxin, PCA. Like PCA, the persistent effects of MDMA on neurotransmitter concentrations seem restricted to the serotonergic system after acute (Schmidt *et al.*, 1986a,b) or multiple drug administration (Stone *et al.*, 1986). *In vitro*, using preloaded rat striatal slices, MDMA and PCA were both potent [^{3}H]5-HT releasing agents while being far less effective at increasing [^{3}H]DA release. In contrast, another neurotoxic amphetamine analog, methamphetamine, was much more potent at inducing [^{3}H]DA release in comparison to [^{3}H]5-HT release (Schmidt *et al.*, 1986b).

The similarities between the neurochemical effects of MDMA and those reported for PCA led us to suggest that the long-term depletions of 5-HT produced by MDMA and PCA occurred through similar mechanisms, *i.e.*, a selective degeneration of serotonergic neurons or nerve terminals (Harvey *et al.*, 1977; Massari *et al.*, 1978). Furthermore, these same similarities indicate that the mechanism of neurotoxicity may be related for the two compounds. In the present study the neurochemical effects of MDMA on the serotonergic system of the rat brain

ABBREVIATIONS: MDMA, methylenedioxymethamphetamine; MDA, methylenedioxyamphetamine; 5-HT, serotonin; PCA, *p*-chloroamphetamine; DA, dopamine.

are characterized further. Evidence is presented indicating that the long-term effects of MDMA are due to a neurotoxic effect on serotonergic neurons and that this neurotoxicity resembles that produced by PCA.

Methods

Drug administration. Male Sprague-Dawley rats (200–250 g) were maintained on a 12-hr light-dark cycle and given free access to food and water. All drugs were dissolved in saline and administered by s.c. injection in a volume of 1 ml/kg. Doses of MDMA-HCl refer to the free base. (±)-MDMA was synthesized from methylenedioxyphenylacetone (piperonylacetone) and methylamine as described by Braun *et al.* (1980). The structure was verified using NMR spectrometry and purity of the product was determined to be greater than 99.5% by elemental analysis. The stereoisomers of MDMA were provided by the National Institute on Drug Abuse (Rockville, MD) and fluoxetine was the generous gift of the Lilly Research Laboratories (Indianapolis, IN).

Determination of cortical 5-HT. Animals were sacrificed at the appropriate times by decapitation and their brains were removed immediately and put on ice. A sample of cerebral cortex corresponding to parietal and frontal cortex was dissected and frozen immediately on dry ice. Samples were stored at −80°C until assayed. Cortical 5-HT was determined using high performance liquid chromatography with electrochemical detection. After being weighed the samples were homogenized in 1 ml of mobile phase containing monochloroacetic acid (0.15 M), EDTA (0.2 mM), heptyl sulfonic acid (1 g/l) and 10% methanol at pH 2.9. N-acetylserotonin (0.1 μg/ml) was added as an internal standard to allow recoveries to be determined. After centrifuging (30,000 × *g*, 15 min) the supernatants were injected directly onto a Waters 5-μm Nov-a-pak C-18 column (Milford, MA). Detection was by means of an ESA Coulochem model 5100A detector (Bedford, MA) using a potential of +0.40 V and a guard potential of +0.05 V. 5-HT was quantitated by comparison with standards of known concentration using a Spectra Physics SP 4270 integrator (San Jose, CA).

Determination of [^{3}H]5-HT uptake. For the uptake experiments animals were sacrificed as described previously and the olfactory bulbs and cerebellum were discarded immediately. The brain was then divided into right and left hemispheres with one side being used to prepare synaptosomes and the other being dissected and frozen for the eventual determination of cortical or whole brain 5-HT concentrations. For the preparation of P_2 synaptosomes each hemibrain was homogenized in 6 ml of 0.32 M sucrose, 1 mM EDTA and centrifuged at 1000 × *g* for 10 min. After discarding the pellet, the supernatant was centrifuged at 15,000 × *g* for 30 min to obtain a P_2 pellet. The pellet was then resuspended in 8 ml of Krebs-Ringer bicarbonate buffer containing (millimolar): NaCl, 118; KCl, 4.85; $CaCl_2$, 2.5; $MgSO_4$, 1.15; KH_2PO_4, 1.15; $NaHCO_3$, 25 and glucose, 1.11 at pH 7.3. All solutions also contained 0.1 mM pargyline (Sigma, Chemical Co., St. Louis, MO). A 100-μl aliquot of this suspension was added to 4 ml of ice-cold Krebs-Ringer bicarbonate buffer containing 0.1 μM [^{3}H]5-HT (New England Nuclear, Boston, MA, specific activity approximately 7.5 Ci/mmol). In experiments to determine the kinetic parameters for 5-HT uptake the concentration of 5-HT was varied while holding the specific activity constant. Total uptake was determined in synaptosomes incubated at 37°C for 10 min in the routine assays and for 5 min in the kinetic assays. Specific uptake was defined as the difference between total uptake and that in synaptosomes maintained on ice during the incubation. At the end of the incubation all samples were filtered rapidly onto Whatman GF/B filters using a Brandel Cell Harvester (Gaithersburg, MD). The filters were washed twice with 5 ml of cold saline and placed in scintillation vials. ACS scintillation cocktail (Amersham Corp., Arlington Heights, IL) was added and the vials were shaken overnight. Samples were counted and corrected for efficiency on a Beckman LS 5801. Synaptosomal protein was determined by the method of Lowry *et al.* (1951).

Statistics. Differences were determined to be statistically significant using the two-tailed Student's *t* test with an acceptable probability being $P < .05$.

Results

Time course of cortical 5-HT depletion by MDMA. To examine the time course of 5-HT depletion by MDMA, rats were administered a single 10 mg/kg (s.c.) injection of the drug and sacrificed at various times thereafter. When 5-HT concentrations were examined in these animals, the results showed two clearly distinguishable phases of 5-HT depletion after MDMA administration. These two phases were most clearly demonstrated in the cerebral cortex. Figure 1 shows cortical results averaged from two identical experiments. As reported previously for striatal 5-HT, there was a rapid and dramatic decrease in cortical 5-HT concentrations immediately after drug administration. By 3 hr cortical 5-HT concentrations had declined to 16% of control. However, between 6 and 24 hr there was a sharp recovery of 5-HT levels such that transmural concentrations had returned control levels 1 day after MDMA administration. Over the next several days, cortical 5-HT concentrations begin to decline again and by 1 week transmural concentrations were reduced to 74% of control ($P < .001$).

Effect of MDMA on whole brain uptake of [^{3}H]5-HT and 5-HT concentrations. The basis of the second phase of 5-HT depletion produced by MDMA was investigated by examining the effect of a single administration of the racemic drug on the uptake of [^{3}H]5-HT by a P_2 synaptosomal preparation from whole brain. Whole brain concentrations of 5-HT were also measured to correlate the two parameters. For these experiments, the dose of MDMA was increased to 20 mg/kg to produce a larger and more consistent reduction in 5-HT concentrations at the 1-week time point. At the time of sacrifice brains from control and treated animals were bisected longitudinally to allow both uptake and 5-HT concentrations to be measured for each animal. As shown in figure 2, a dose of 10 mg/kg of MDMA decreased whole brain 5-HT concentration to approximately 65% of control after 7 days. In the same animals, the uptake of [^{3}H]5-HT by whole brain synaptosomes was reduced to approximately 50% of control.

Stereochemical specificity of the acute and long-term effects of MDMA. We have reported previously that either stereoisomer of MDMA can produce the acute depletion

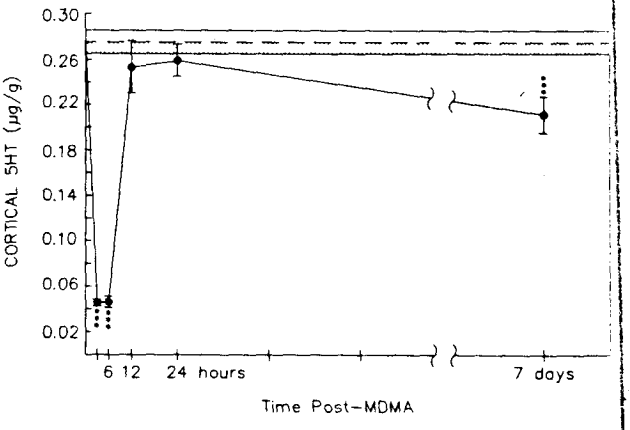


Fig. 1. Time course of the changes in cortical 5-HT concentrations after the administration of a single dose of (±)-MDMA (10 mg/kg s.c.). Results are the averages from two complete experiments with each point being the mean ± S.E.M. for eight or more animals. ***$P < .001$ *vs.* control by the two-tailed Student's *t* test.

Fig. 2. Effect of (±)-MDMA (20 mg/kg s.c.) on whole brain 5-HT (left) and synaptosomal [^{3}H] 5-HT uptake (right) 1 week after drug administration. Each point is the mean ± S.E.M. for five animals. $^{**}P < .01$ *vs.* control by the two-tailed Student's *t* test.

striatal 5-HT and 5-hydroxyindoleacetic acid observed 3 hr after drug administration but that the two stereoisomers did differ in their long-term effects on striatal indole concentrations (Schmidt *et al.*, 1986b). As shown in figure 3, very similar results were observed for the effects of the two enantiomers on cortical 5-HT concentrations. Acutely, both (+)- and (−)-MDMA (10 or 20 mg/kg) reduced cortical 5-HT to between 10 and 20% of control 3 hr after drug administration (fig. 3A). These experiments were then repeated at the 1-week time point to compare the effects of the two stereoisomers on cortical 5-HT concentrations and on the uptake of [^{3}H]5-HT by a P_2 preparation. (+)-MDMA, but not the (−)-stereoisomer, produced a dose-dependent reduction in cortical 5-HT concentrations 1 week after drug administration (fig. 3B). At the higher dose of MDMA (20 mg/kg), this decrease in 5-HT concentrations was correlated with an approximately 50% reduction in [^{3}H]5-HT uptake. (−)-MDMA was without effect on [^{3}H]5-HT uptake.

Kinetic evaluation of the MDMA-induced decrease in [^{3}H]5-HT uptake. To determine if the decrease in 5-HT uptake measured in the synaptosome preparation was due to a loss of 5-HT uptake sites or a change in the affinity of the carrier for the amine, the kinetic parameters for 5-HT uptake were evaluated in control and MDMA-treated animals. As in the previous uptake experiments, rats received (+)-MDMA (20 mg/kg s.c.) or saline 7 days before sacrifice and P_2 synaptosomes were prepared from hemibrains from each animal. Synaptosomal 5-HT uptake was then determined at substrate concentrations from 0.0125 to 0.1 μM during 5-min incubations. After correction for protein, the data were fitted by linear regression to a straight line of velocity *vs.* velocity/[S] according to the method of Eadie and Hofstee (Fersht, 1977). In such, a plot V_{max} is given by the *y*-intercept while the slope is equal to

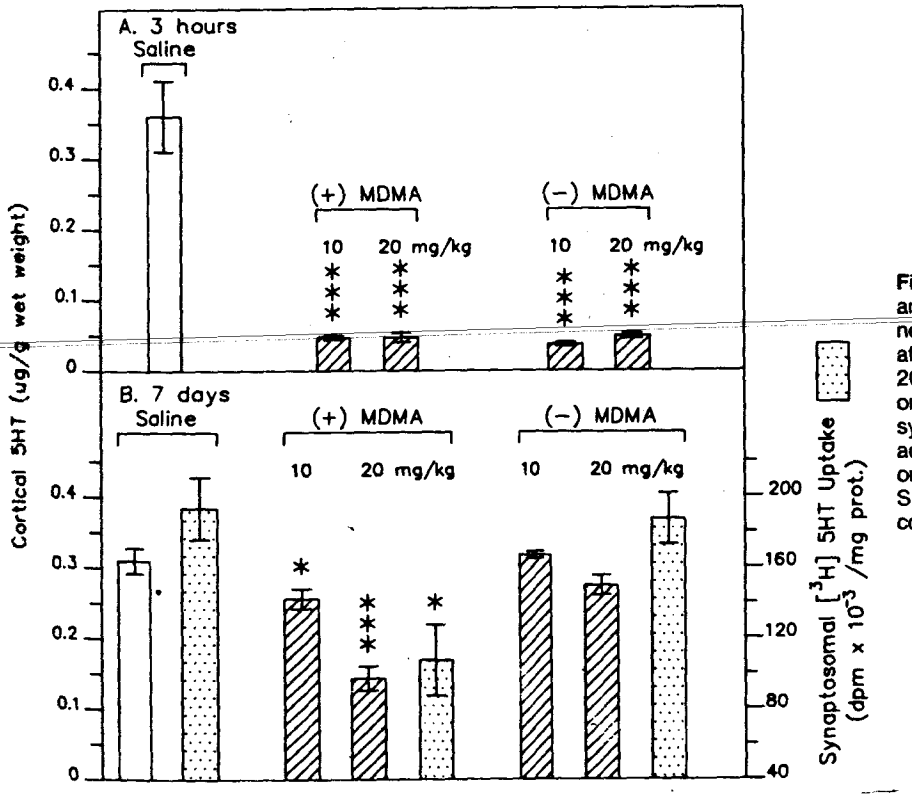


Fig. 3. Stereochemical requirements for the acute and long-term effects of MDMA on cortical serotonergic neurons. A: Depletion of cortical 5-HT 3 hr after the administration of (+)- or (−)-MDMA (10 or 20 mg/kg s.c.). B: Effect of (+)- and (−)-MDMA (10 or 20 mg/kg, s.c.) on cortical 5-HT and whole brain synaptosomal [^{3}H]5-HT uptake 1 week after drug administration. Uptake experiments were performed only at the higher dose. All points are the mean ± S.E.M. for five animals. $^{*}P < .05$, $^{***}P < .001$ *vs.* control by the two-tailed Student's *t* test.

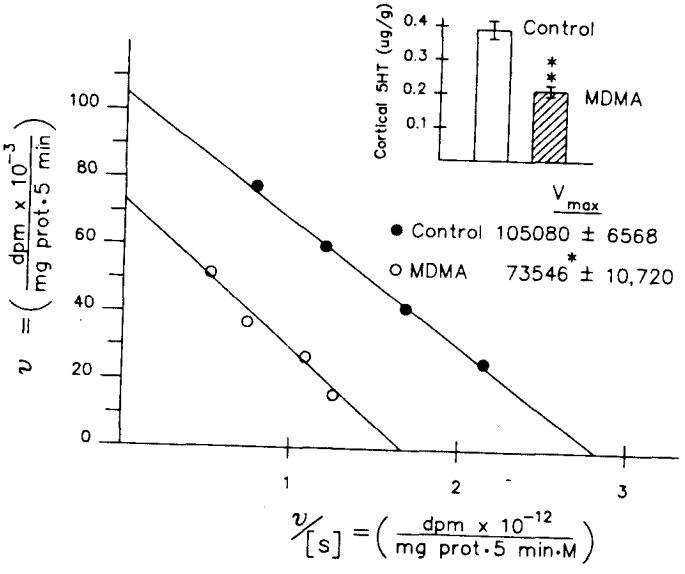
the $-K_m$. Brain regions from the remaining hemisphere were used to determine the degree of 5-HT depletion produced by MDMA.

As seen from the insert in figure 4, cortical 5-HT was reduced by approximately 50% in this experiment 1 week after the administration of (+)-MDMA. The data in the lower one-half of the figure demonstrate that there was also a significant decrease in [^{3}H]5-HT uptake by synaptosomes prepared from the MDMA-treated animals. Comparison of the y-intercepts and the slopes of the two lines indicates that this decrease is due to 30% reduction in the V_{max} for uptake with no significant change in the K_m.

Time-dependent blockade of the long-term effect of MDMA on cortical 5-HT by fluoxetine. The acute depletion of rat brain 5-HT by MDMA is blocked by the simultaneous administration of the 5-HT uptake inhibitor, citalopram (Schmidt *et al.*, 1986b). To determine if the long-term or second phase of MDMA-induced 5-HT depletion was also sensitive to uptake inhibition, similar experiments were performed using fluoxetine, another selective 5-HT uptake inhibitor (Fuller *et al.*, 1974). In the first experiment, fluoxetine (5 mg/kg s.c.) was administered simultaneously with (±)-MDMA (20 mg/kg s.c.) and the animals were sacrificed 1 week later. MDMA alone reduced cortical 5-HT to 56 ± 6% of control and fluoxetine itself was without significant effect (data not shown). When the two drugs were coadministered, cortical 5-HT values were maintained at 102 ± 9% of control. In a second experiment, rats were again administered (±)-MDMA at the 20 mg/kg dose followed by a single injection of fluoxetine (5 mg/kg) at 3, 6 and 12 hr after the MDMA. Fluoxetine controls were run at all time points. The results of this experiment are shown in figure 5 with the effect of MDMA being expressed as a percentage of the appropriate control (*i.e.*, percentage of saline control in the case of MDMA alone or as a percentage of the fluoxetine control at each time point. Absolute values for the controls are provided in the figure legend. The data for the simultaneous administration of MDMA and fluoxetine were included from the first experiment for illustrative purposes. As described already, coadministration of fluoxetine completely blocked the depletion of cortical 5-HT, which occurs 1 week after a single administration of MDMA. However, the administration of fluoxetine 3 hr after MDMA was still sufficient to completely antagonize the effect of MDMA as measured 1 week later. By 6 hr postMDMA, the administration of fluoxetine no longer completely blocked the 5-HT depletion as cortical 5-HT concentrations were reduced to 72% of control. This still represented significant protection since in this experiment MDMA alone reduced 5-HT concentrations to 39% of control. In animals treated with fluoxetine 12 hr after MDMA, cortical 5-HT concentrations were depressed to 43% of control at 1 week, a value no longer different than that observed for animals treated with MDMA alone.

Discussion

The results clearly demonstrate that MDMA has two distinct influences on the serotonergic system of the rat brain, both of which are manifested by a significant decrease in the concentration of 5-HT. Whereas the first effect of MDMA appears to produce a reversible depletion of 5-HT, the second is due to a neurotoxic effect of the drug on serotonergic neurons or nerve terminals. As demonstrated by the results of the experiments shown in figures 1 and 5, these two effects of MDMA can be separated temporally into an early and a later phase. The depletion observed at 3 hr is due almost completely to the acute effect of the drug inasmuch as it is reversed completely by 24 hr and the long-term depletion can be blocked independently by fluoxetine even though cortical 5-HT concentrations may be reduced by greater than 80% (figs. 1 and 3A). Therefore, inhibition of the uptake system by 3-hr postMDMA can still completely protect the serotonergic neurons from the later effects of MDMA. At 6 hr after MDMA the irreversible effects of the drug have progressed to the extent that the administration of fluoxetine at this point blocks only an approximate 50% of the depletion of 5-HT observed at 1 week in the absence of the uptake inhibitor. By 12 hr the steps required for the drug's irreversible effects on 5-HT concentrations have been completed even though the results for animals treated with 10 mg/kg of MDMA suggest 5-HT concentrations have returned temporarily to near control values at this time (compare figs. 1 and 5). Although these results suggest that the immediate depletion of 5-HT concentrations by MDMA can occur independently of



	V_{max}	K_m
● Control	105080 ± 6568	.0373 ± .0026uM(4)
○ MDMA	73546* ± 10,720	.0440 ± .0040uM(4)

Fig. 4. Eadie-Hofstee plot of synaptosomal [^{3}H]5-HT uptake for control and MDMA-treated animals. (+)-MDMA (20 mg/kg s.c.) was administered 1 week before the experiment. Insert: Cortical 5-HT concentrations for animals used in the uptake experiment. Each point in both figures is the mean for four animals. *$P < .05$, **$P < .01$ *vs.* control by the two-tailed Student's *t* test.

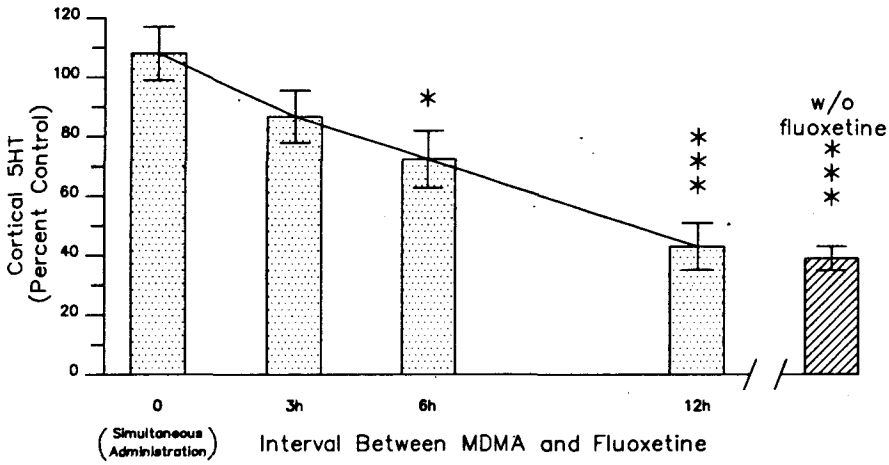


Fig. 5. Time-dependent effect of fluoxetine on the long-term depletion of cortical 5-HT by MDMA. (±)-MDMA (20 mg/kg s.c.) was administered at time zero and fluoxetine (5 mg/kg s.c.) was administered either simultaneously or 3, 6 and 12 hr later. Animals were sacrificed 1 week after MDMA. All values are expressed as a percentage of the appropriate control. Absolute values were (microgram per gram): saline, 0.33 ± 0.02; fluoxetine, 0.33 ± 0.02; 3 hr fluoxetine, 0.35 ± 0.01; 6 hr fluoxetine, 0.36 ± 0.02; 12 hr fluoxetine, 0.39 ± 0.02. Each point is the mean for five animals ± S.E.M. $^{*}P < .05$, $^{***}P < .001$ *vs.* saline control by the two-tailed Student's *t* test.

any long-term effects it is not known if the immediate depletion is somehow required for the long-term effects of MDMA to occur.

The two phases of 5-HT depletion are also differentiated by their stereoselectivity (fig. 3). Whereas both stereoisomers of the drug caused the reduction in 5-HT concentrations measured at 3 hr, only (+)-MDMA produced the depletion of cortical and striatal (Schmidt *et al.*, 1986b) 5-HT when tissue concentrations of the amine were determined 1 week after drug administration. The long-term or persistent effect of MDMA on serotonergic neurons is therefore a property of the (+)-stereoisomer. This statement is supported by the fact that only the (+)-stereoisomer of MDMA reduced the uptake of [^{3}H]5-HT by whole brain synaptosomes prepared from animals treated 1 week earlier with (+)- or (−)-MDMA.

The differences between the two effects of MDMA on serotonergic neurons reflect the differences in their mechanisms. The acute changes in 5-HT concentration caused by the drug are reversible and therefore involve changes in transmitter turnover. Although (+)-MDMA is more potent than the (−)-stereoisomer for inducing 5-HT release from either synaptosomes (Nichols *et al.*, 1982) or striatal slices (Schmidt *et al.*, 1986b) both stereoisomers are effective 5-HT-releasing agents *in vitro* and are clearly capable of affecting transmitter turnover at the doses used in this study. Data from our previous work suggest that beyond an increase in transmitter release, both stereoisomers of MDMA probably also directly inhibit reuptake mechanisms and monoamine oxidase activity (Schmidt *et al.*, 1986a,b). It is likely that all these actions contribute to the depression in 5-HT concentrations observed shortly after MDMA administration, *i.e.*, increased release coupled with reduced transmitter synthesis due either to end-product inhibition or 5-HT autoreceptor activation.

The basis of the second phase of MDMA-induced 5-HT depletion is clearly demonstrated by the results from the synaptosomal uptake experiments, which confirm a loss in 5-HT uptake sites. This likely corresponds to a degeneration of 5-HT nerve terminals and a concomitant decrease in the concentration of 5-HT. Although the pattern of this depletion and the structural similarities between MDMA and a number of neurotoxic amphetamine analogs previously led us to suggest that MDMA could be neurotoxic, the results from the uptake studies presented here provide biochemical evidence of terminal damage. Kinetic analysis of 5-HT uptake in animals treated with MDMA revealed a significant decrease in the V_{max} of the uptake carrier with no change in the affinity for 5-HT, indicating that the decrease in 5-HT uptake is due to a loss in uptake sites and not merely a change in the affinity of the uptake carrier for 5-HT.

An interesting feature of the neurotoxic effect of MDMA is the fact that it is primarily due to the (+)-stereoisomer. Because the acute effects of the drug on the serotonergic neuron do not have any stereochemical requirements this suggests that the development of the persistent or neurotoxic effects of the drug occur independently of the acute effects and that the acute and massive depletion of 5-HT by (+)- or (−)-MDMA is not necessarily a prelude to or the cause of the eventual neurotoxic response. A possible explanation is that the acute effect of MDMA on brain 5-HT concentrations is due to the amphetamine-like nature of MDMA, whereas the neurotoxicity may be due to the cumulative toxic effect of a metabolite of the drug. The generation of this hypothetical metabolite must occur during the first 12 hr after drug administration inasmuch as the long-term alterations produced by MDMA are largely irreversible after that time. The ability of fluoxetine to significantly interfere with the neurotoxicity at a time when the acute effects of the drug are at a maximum further suggests that this metabolite may also be a substrate for the 5-HT uptake carrier because only the neurotoxic phase can be antagonized at this point. We have demonstrated previously that [^{3}H]MDMA is not accumulated by rat brain synaptosomes to any large extent, suggesting the compound probably enters neurons principally by diffusion (Schmidt *et al.*, 1986b). This means that inhibition of the uptake carrier would not interfere with MDMA itself entering the neuron. Although the 5-HT uptake inhibitor, citalopram, can block both the acute depletion of 5-HT by MDMA *in vivo* as well as the MDMA-induced release of [^{3}H]5-HT from striatal slices *in vitro* (Schmidt *et al.*, 1986b) both the *in vivo* and *in vitro* effect of the inhibitor can be explained as interference with the carrier-mediated efflux of 5-HT induced by MDMA. Similar findings have been reported for other amphetamine analogs (Fuller and Snoddy, 1979; Schmidt and Gibb, 1985). Distinct from these results, the ability of fluoxetine to block the long-term effects of MDMA even when administered 3 hr after MDMA points to a second carrier-mediated event. Metabolism of a lipophilic compound such as MDMA to a compound requiring carrier transport is not surprising in view of the fact that most xenobiotic metabolism leads to compounds of greater hydrophilicity, hence, less membrane penetrance, to enhance their elimination (Goldstein *et al.*, 1974). Why such a

metabolite should have an affinity for the 5-HT carrier is less apparent, although DA (Schmidt and Lovenberg, 1985; Kelly *et al.*, 1985) as well as a number of other phenethylamines (Raiteri *et al.*, 1977) related to MDMA have been shown to have an affinity for the 5-HT uptake carrier. The selectivity of the neurotoxic effect of MDMA for serotonergic neurons also makes it tempting to speculate on the possible cyclization of the isopropylamine side-chain of MDMA to yield an indolic structure which after demethylenation would be structurally related to selective serotonergic neurotoxins such as 5,7-dihydroxytryptamine. A similar conversion has been proposed as an explanation for the selective neurotoxicity of PCA (Gal, 1976). Further work on this possibility is now in progress.

We have observed previously that MDMA and the well established neurotoxin, PCA, are very similar in a number of their acute neurochemical effects (Schmidt *et al.*, 1986a). The data presented here provide additional examples of the similarities between the two drugs. For example, both MDMA and PCA have acute, reversible effects on 5-HT concentrations with the irreversible effects developing only later (Fuller *et al.*, 1975; Ross, 1976; Sanders-Bush and Steranka, 1978). Both drugs are potent and relatively selective [^{3}H]5-HT releasing agents when compared *in vitro* to an agent such as methamphetamine, which is in turn more potent at inducing [^{3}H]DA release. In keeping with this, the effects of both drugs on neurochemical parameters of the dopaminergic system *in vivo* are minor and short-lived in comparison to effects on the serotonergic system. Finally, the stereochemical requirements for the neurochemical effects of both drugs are identical in that whereas both stereoisomers of the drugs will produce the acute, reversible depletion of 5-HT, their (+)-enantiomers are more potent at producing the long-term decrease in 5-HT concentrations that is associated with neurotoxicity (Sekerke *et al.*, 1975). A number of investigators have reported evidence which suggested that the neurotoxicity produced by PCA is in fact due to the formation of a chemically reactive intermediate (Sherman *et al.*, 1975; Ames *et al.*, 1977). This information coupled with the number of similarities between the neurochemical effects of MDMA and PCA lends additional support to the hypothesis that a reactive metabolite is also responsible for MDMA-induced neurotoxicity.

Although it is difficult to extrapolate from animal studies to the human situation, some comment about the risk to human MDMA users seems appropriate in view of the widespread abuse of MDMA as a recreational drug. It is first important to point out that the parenteral doses used in this study are approximately 4 to 8 times the human oral dose (Shulgin and Nichols, 1978). There is also evidence to suggest that drugs such as MDMA and MDA may be subject to significant first pass metabolism inasmuch as hepatic demethylenation of MDA is a major route of metabolism in the rat, dog and monkey (Glennon, 1984). This would mean that the doses used in this study may be even further from those to which a user taking the drug p.o. is normally exposed. However, all the effects described in this study were produced with a single administration of MDMA; consequently, the possible cumulative effects of multiple MDMA exposures remain to be evaluated. Finally, one should consider the lesson learned from 1-methyl-4-phenyltetrahydropyridine, which has very little effect in rats (Enz *et al.*, 1984; Boyce *et al.*, 1984) even at high doses but produces Parkinsonism in primates including man by a selective lesion of the nigrostriatal DA pathway at very low doses (Burns *et al.*, 1983). The possibility that humans might likewise be more sensitive to MDMA-induced neurotoxicity must not be overlooked.

In conclusion, this study presents evidence that MDMA has complex effects on serotonergic neurons beginning with a rapid, but reversible, depletion of transmitter and culminating in a neurotoxic effect at the nerve terminal. The results suggest a number of pharmacological similarities between MDMA and the neurotoxin, PCA, and confirm that there are toxicological grounds for concern over the widespread abuse of MDMA and related compounds.

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