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Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs

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The acute and long-term neurochemical effects of three methylenedioxyamphetamine analogs were examined in the serotonergic system of the rat brain. Methylenedioxymethamphetamine as well as its N-desmethyl and N-ethyl derivatives depleted cortical serotonin (5HT) concentrations to less than 30% of control 3 h after drug administration. All three compounds were also very similar in their effects on [^{3}H]5HT release from superfused rat striatal slices. Increasing the size of the N-alkyl substituent did appear to reduce the potency of the agent for inducing [^{3}H]dopamine release. One week following drug administration cortical 5HT concentrations had returned to control levels in animals treated with the N-ethyl derivative while the other two analogs produced persistent depletions in transmitter concentrations. The effects of the latter two drugs were correlated with a significant decrease in whole brain synaptosomal [^{3}H]5HT uptake indicating serotonergic nerve terminal damage. N-ethyl-methylenedioxyamphetamine had no effect on synaptosomal 5HT uptake at one week. The (+) stereoisomer of methylenedioxyamphetamine was slightly more potent than the (−) enantiomer at producing the long-term 5HT depletion as previously shown for its N-methyl derivative suggesting similar mechanisms may be responsible for their neurotoxic effects.

Methylenedioxyamphetamine; Amphetamine; Neurotoxicity; Serotonin; Hallucinogen

1. Introduction

The phenylisopropylamines form a large and well-studied class of psychoactive drugs with a wide range of pharmacological effects. Modification of the basic amphetamine structure by ring substitution of one or more methoxy groups converts agents which are primarily central stimulants to drugs which are commonly classified as hallucinogenic or psychedelic. Ring substitution of a methylenedioxy function yields a unique group of compounds which seem to possess pharmacological properties of both the amphetamines and their hallucinogenic derivatives. Although these compounds have been known for some time, relatively little is understood concerning their neurochemical actions or the mechanism of their behavioral effects. Attention has recently been focused on this group due to the increase in the illicit use of methylenedioxymethamphetamine (MDMA) as a recreational drug. Both MDMA and its parent compound, methylenedioxyamphetamine (MDA) are classified as Schedule I compounds by the Federal Drug Enforcement Agency. MDA itself has been a relatively popular street drug during the previous 20 years. MDA can produce a number of serious side-effects related to its strong amphetamine-like character and there have been a number of fatal cases of overdose with the drug (Cimbura, 1972; Lukaszewski, 1979; Simpson and Rumack, 1981). N-methylation of MDA to MDMA produces a compound of similar psychotomimetic potency with few resultant side-effects (Shulgin, 1978). With the 1985 banning of MDMA, another member of this series has appeared on the illicit drug market, N-ethyl-methylenedioxyamphetamine (MDE). Currently, MDE is not a restricted compound.

MDMA differs from the central stimulant methamphetamine only in the addition of the methylenedioxy group on the phenyl ring. The neurotoxic effects of high doses of methamphetamine on catecholaminergic and serotonergic systems in the rat brain has been demonstrated by a number of investigators (Hotchkiss and Gibb, 1980; Wagner et al., 1980; Trulson and Trulson, 1982; Schmidt et al., 1985). The structural similarities between MDMA and methamphetamine led us to investigate whether a similar neurotoxicity would be observed with MDMA in rats. Like several other neurotoxic amphetamine derivatives, MDMA was found to produce both an immediate and long-term depletion of serotonin (5HT) in the rat brain. The depletion of 5HT was a selective effect in that dopamine (DA) concentrations were not similarly altered by MDMA administration (Schmidt et al., 1986). This is consistent with in vitro results showing MDMA to be more potent at releasing 5HT than DA from striatal slices (Schmidt et al., in press). The immediate and long-term effects of MDMA on 5HT concentrations appear to be mediated by separate mechanisms since they can be separated temporally and with respect to their stereochemical requirements. While either stereoisomer of MDMA produced an acute depletion of striatal (Schmidt et al., in press) or cortical (Schmidt, in press) 5HT concentrations only the (+) stereoisomer produced the long-term depletion of transmitter concentrations observed one week after drug administration. The long-term depletion of 5HT produced by (+)-MDMA has been shown to be associated with a significant decrease in the V_{max} for synaptosomal [^{3}H]5HT uptake indicating the loss of, or damage to, serotonergic nerve terminals (Schmidt, in press). All of the neurochemical effects outlined for MDMA are very similar to those of the well-known serotonergic neurotoxin, p-chloroamphetamine (PCA) which prompted the suggestion that the two agents may have similar mechanism of neurotoxicity.

The purpose of the current investigation was to compare the neurochemical effects of MDMA with those of its analogs MDA and MDE to further examine the potential neurotoxicity of this group of compounds.

2. Materials and methods

2.1. Drug administration

Male Sprague-Dawley rats (200-250 g) were maintained on a 12 h light-dark cycle and given free access to food and water. All drugs were dissolved in saline and administered by subcutaneous (s.c.) injection in a volume of 1 ml/kg. Doses of the three drugs 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 and purity of the product after recrystallization was determined to be greater than 99.5% by elemental analysis. The resolved stereoisomers of MDA, (±)-MDA and (±)-MDE were provided by the National Institute on Drug Abuse. Drug doses were selected based on previous experiments comparing the immediate and long-term effects of MDMA on cortical 5HT concentrations (Schmidt, in press).

2.2. Determination of cortical 5HT

Animals were killed at the appropriate times by decapitation and their brains were immediately removed on ice. A sample of cerebral cortex corresponding to parietal and frontal cortex was dissected and immediately frozen on dry ice. Samples were stored at −80°C until assayed. Cortical 5HT concentrations were 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. 5HT was quantitated by comparison with standards of

known concentration using a Spectra Physics SP 4270 integrator (San Jose, CA).

2.3. Release studies

All superfusion experiments were conducted using preloaded rat striatal slices to allow comparison of both [^{3}H]DA or [^{3}H]5HT release. For each experiment two male Sprague-Dawley rats (200-250 g) were killed by decapitation, their brains immediately removed 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°C 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, glucose 11.1 at pH 7.3. All solutions also contained 10^{-4} M pargyline and were constantly bubbled with 95% O_2-5% CO_2. [^{3}H]DA (New England Nuclear, Boston, MA, 7.5 Ci/mmol) or [^{3}H]5HT (NEN, 7.5 Ci/mmol) 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 (Schmidt and Gibb, 1985) 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 cocktail (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.

2.4. Determination of [^{3}H]5HT uptake

For the uptake experiments animals were killed as described previously and the olfactory bulbs and cerebellum were immediately discarded. 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 5HT 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 1 000 × 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 KRB. A 100 μl aliquot of this suspension was added to 4 ml of ice cold KRB containing 0.1 μM [^{3}H]5HT (NEN, 7.5 Ci/mmol). Total uptake was determined in synaptosomes incubated at 37°C for 10 min. 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 rapidly filtered 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, Arlington Hts., 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).

3. Results

3.1. Acute effect of methylenedioxyamphetamines on cortical 5HT

Rats were administered either MDA, MDMA or MDE at 10 or 20 mg/kg by s.c. injection and killed 3 h later. All three drugs caused massive decreases in the concentration of cortical 5HT to 20% or less of control values as shown in fig. 1. There were no major differences in the degree of depletion produced by any of the drugs at the doses used here. These doses were apparently at near maximum for both MDA and MDMA in producing this effect as either 10 or 20 mg/kg reduced 5HT concentrations to a similar extent. However, the higher dose of MDE did produce a

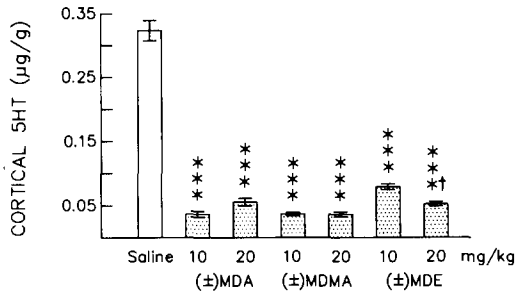

Fig. 1. Acute effect of racemic methylenedioxyphenylisopropylamines on cortical 5HT concentrations. Rats were administered a single injection of the drugs (10 or 20 mg/kg s.c.) 3 h prior to killing. Results are the means ± S.E.M. for n = 5. *** $P < 0.001$ vs. control; $^{+}$ $P < 0.05$ vs. 10 mg/kg MDE by the two-tailed Student's t-test.

significantly greater depletion of cortical 5HT concentrations than the 10 mg/kg dose, although the difference was not large.

3.2. *Release of monoamines by methylenedioxyamphetamines in vitro*

The three methylenedioxyamphetamine analogs were compared for their ability to release ^{3}H-monoamines from rat striatal slices superfused in vitro. As shown in fig. 2 (left) all three drugs caused a similar concentration-dependent release of [^{3}H]5HT. Only at the highest drug concentration was there any significant difference in the release produced by the compounds. At 10^{-5} M, MDA produced significantly greater release of 5HT than either MDMA or MDE. Differences between the three drugs were observed when their effect on the release of [^{3}H]DA was investigated (fig. 2, right). Both MDA and MDMA produced significantly greater release of [^{3}H]DA from striatal slices than did MDE at all concentrations tested. MDA was also more potent than MDMA at inducing [^{3}H]DA release at all but the lowest concentration of drug. In comparing the data from both parts of fig. 2 it is apparent that all three compounds are more potent as 5HT-releasing agents than as DA-releasing agents. Measurable release of [^{3}H]5HT is first detected at 10^{-7} M while the concentrations of the drugs had to be increased 10-fold to initiate [^{3}H]DA release.

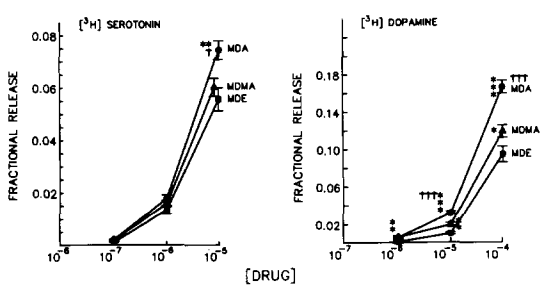

Fig. 2. Comparison of [^{3}H]5HT (left) and [^{3}H]DA release (right) from superfused rat striatal slices by methylenedioxyphenylisopropylamines. Release is expressed as fraction of radioactivity contained in the slices released by a 5 min pulse of drug. Results are the means ± S.E.M. for n = 8. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. MDE and † $P < 0.05$, ††† $P < 0.001$ vs. MDMA by the two-tailed Student's t-test.

3.3. *Long-term effect of methylenedioxyamphetamines on cortical 5HT concentrations*

Experiments similar to those examining the immediate effects of the three analogs were repeated to investigate the persistent effects of the drugs. Changes in cortical 5HT concentrations were measured one week after the administration of a single dose of MDA, MDMA or MDE at either 10 or 20 mg/kg. Both MDA and MDMA produced significant reductions in cortical 5HT concentrations one week after drug administration (fig. 3). This effect was most dramatic for MDA which reduced 5HT concentrations to 40% of control or less at both doses. Although the higher dose of MDMA

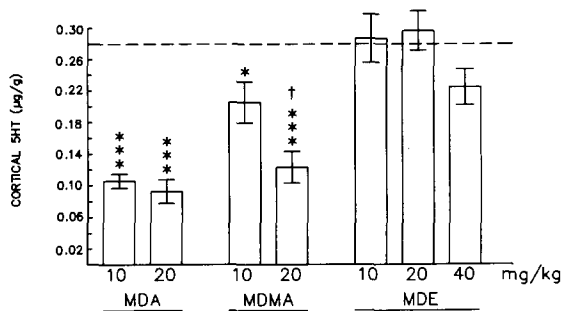

Fig. 3. Long-term effect of racemic methylenedioxyphenylisopropylamines on cortical 5HT concentrations. Rats were administered a single injection of the drugs at each dose 7 days prior to killing. Results are the means ± S.E.M. for n = 5. * $P < 0.05$; *** $P < 0.001$ vs. control (dashed line); † $P < 0.05$ vs. 10 mg/kg MDMA by the two-tailed Student's t-test.

produced an effect equivalent to that of MDA, the 10 mg/kg dose only decreased 5HT concentrations to approximately 74% of control. This indicates that a dose of 10 mg/kg may be near maximum for this effect of MDA while 20 mg/kg is required for MDMA to produce a maximum depletion. In contrast to the effects observed for MDA and MDMA, the N-ethyl derivative, MDE, did not cause any significant reductions in cortical 5HT concentrations one week after drug administration even when the dose was increased to 40 mg/kg in a separate experiment.

3.4. Long-term effect of methylenedioxyamphetamines on whole brain synaptosomal 5HT uptake

Hemibrains from animals given the 20 mg/kg dose of either MDA, MDMA or MDE were also used to determine the effect of the drugs on the uptake of [^{3}H]5HT by a P_2 synaptosomal preparation. These results are shown in fig. 4 together with the cortical 5HT values for the same animals taken from fig. 3 to allow comparison of the two

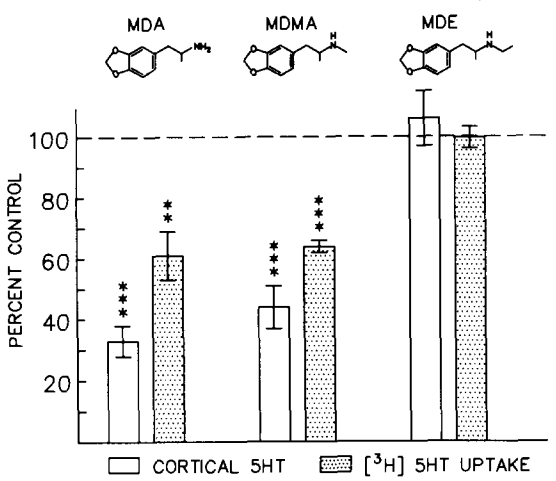

Fig. 4. Long-term effects of racemic methylenedioxyphenylisopropylamines on synaptosomal [^{3}H]5HT uptake. Rats were administered a single injection of the drugs (20 mg/kg s.c.) 7 day prior to killing. Cortical 5HT concentrations for the same animals were taken from fig. 3 for comparison of the two parameters. All values are the means ± S.E.M. for n = 5 expressed as a percent of saline controls. Absolute values were 0.280 ± 0.017 μg/g for cortical 5HT and 71 565 ± 3 680 d.p.m./5 min·mg protein for uptake. ** $P < 0.01$; *** $P < 0.001$ vs. control by the two-tailed Student's t-test.

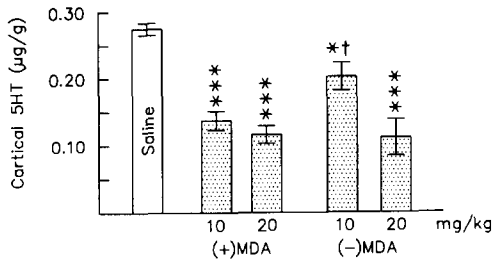

Fig. 5. Comparison of the long-term effects of the stereoisomers of MDA on cortical 5HT concentrations. Rats were administered a single injection of each stereoisomer 7 days prior to killing. Results are the means ± S.E.M. for n = 5. * $P < 0.05$; *** $P < 0.001$ vs. control and † $P < 0.05$ vs. 10 mg/kg (+)-MDA by the two-tailed Student's t-test.

parameters. A single administration of either MDA or MDMA resulted in a highly significant decrease in synaptosomal [^{3}H]5HT uptake one week after drug administration. Both drugs reduced synaptosomal [^{3}H]5HT uptake to between 60 and 70% of control. In agreement with the lack of any effect on cortical 5HT, 20 mg/kg of MDE did not alter [^{3}H]5HT uptake.

3.5. Stereochemical specificity of the long-term depletion of cortical 5HT by MDA

To determine if the long-term depletion of 5HT by MDA had any stereochemical requirements, rats were administered either (+)- or (−)-MDA at both 10 and 20 mg/kg and killed one week later for the determination of cortical 5HT concentrations. The results of these experiments are shown in fig. 5. Both stereoisomers of MDA produced a significant depletion of cortical 5HT concentrations one week after drug administration. At the lower dose of 10 mg/kg, (+)-MDA was significantly more potent than (−)-MDA in this regard. Both stereoisomers of MDA produced equivalent depletions in cortical 5HT concentrations at the higher dose of 20 mg/kg. These latter results were confirmed in a second experiment.

4. Discussion

All three methylenedioxyamphetamine analogs caused a similar immediate depletion of cortical

5HT concentrations 3 h after their acute administration to rats. At the two doses used in this study there was no major difference in this regard between the compounds. In the case of MDMA, we have suggested that this immediate depletion of 5HT is due to a combination of increased transmitter release coincident with a decrease in transmitter synthesis (Schmidt et al., in press). The in vitro release data showing all three agents have similar potency at inducing 5HT release would therefore be consistent with the very similar acute effect of the drugs on 5HT concentrations.

Differences in the three drugs became apparent when the long-term effects of the agents on the serotonergic system were examined. Either MDA or MDMA can produce a persistent depletion of rat brain 5HT concentrations after a single administration of drug. For both drugs this decrease in 5HT concentrations is associated with a loss in 5HT uptake sites, a biochemical marker of neurotoxicity. Ricaurte et al. (1985) demonstrated similar effects for MDA after a four day regimen of the drug. Unlike the first two agents of the series, a single injection of the N-ethyl analog, MDE, did not produce the long-term depletion of cortical 5HT concentrations even when the dose of the drug was increased to 40 mg/kg. This is four times the dose at which significant long-term depletions of 5HT can be observed with either MDA or MDMA. Consistent with its lack of effect on cortical 5HT one week after drug administraton, MDE did not alter the synaptosomal uptake of [^{3}H]5HT. Therefore, although all three drugs produced the acute depletion in cortical 5HT concentrations, only MDA and MDMA gave rise to a neurotoxic effect. This provides further evidence that acute and long-term phases of 5HT depletion are distinct and due to different mechanisms. Although these and other data (Schmidt et al., in press; Schmidt, in press) clearly show that the acute depletion of 5HT by these drugs is not always a prelude to the development of neurotoxicity, it is not clear if an acute depletion of 5HT is necessary to the development of the neurotoxicity. In other words, the acute phase of 5HT depletion can occur without neurotoxicity developing but the reverse may not be true. The administration of an inhibitor of 5HT uptake such as fluoxetine 3 h after the administration of MDMA, when the acute depletion of 5HT should already be maximum, can still completely block the long-term depletion of transmitter (Schmidt, in press). However, simultaneous administration of the inhibitor with MDMA to block the acute effect of MDMA also blocks the long-term effects of the drug (Schmidt et al., in press; Schmidt, in press). The relationship between the immediate and long-term effects of MDA and MDMA on serotonergic neurons remains to be clarified.

Several investigators have provided evidence for the involvement of a toxic metabolite of PCA in its long-term effects on serotonergic neurons (Sherman et al., 1975; Ames et al., 1977). Although the mechanism of MDMA neurotoxicity is unknown, we have previously suggested it may be similar to that proposed for PCA. This hypothesis was based upon a number of neurochemical similarities between the two drugs (Schmidt et al., in press). The structural and neurochemical similarities between MDA and MDMA make it very likely that the neurotoxicity produced by both drugs may also be due to a similar mechanism and possibly an identical metabolite. Like PCA (Sekerke et al., 1975), the long-term effect of both MDMA (Schmidt et al., in press; Schmidt, in press) and MDA (this paper) on 5HT concentrations is primarily a property of the (+) stereoisomer of the drug. The difference between the two stereoisomers of MDA was less pronounced than that observed with (+)- and (−)-MDMA since neither 10 nor 20 mg/kg (−)-MDMA produce significant depletion of cortical 5HT one week after a single dose of the drug (Schmidt, in press). Nevertheless, these results are not inconsistent with the conversion of MDMA to MDA in vivo with MDA possibly being the penultimate neurotoxin. Experiments are now in progress to determine if peripheral metabolism, particularly N-demethylation, is required for the long-term effects of MDMA on the serotonergic system.

Our results with the stereoisomers of MDA indicate that the neurotoxicity of the drug is not related to its hallucinogenic effect since the latter property is reported to reside exclusively in the (−) enantiomer. This is in contrast to the situation with MDMA in which the (+) stereoisomer

is reported to produce both the psychedelic effects (Anderson et al., 1978) and neurotoxicity (Schmidt, in press). The mechanism responsible for behavioral effects of the two drugs is therefore presumed to be different. In support of this there is reportedly no cross-tolerance between the two drugs (Anderson et al., 1978). Furthermore, Lyons et al. (1986) recently reported that the psychoactive enantiomer of MDMA had a very low affinity for the $5HT_2$ receptor in contrast to that of the psychoactive enantiomer of MDA. This suggests that MDMA does not work though a direct interaction at a 5HT receptor as has been proposed for numerous other hallucinogenic phenethylamines (Glennon et al., 1984). These results imply a novel mechanism may be responsible for the psychedelic effects of MDMA.

The observation that (+)-MDMA was more potent than the (−) stereoisomer at releasing [^{3}H]5HT from rat brain synaptosomes led to the proposal that the release of endogenous 5HT might be involved in the drug's psychedelic effects (Nichols et al., 1982). Indeed, numerous investigators have described the hallucinogenic phenethylamines as a continuum ranging from drugs with direct agonist properties at 5HT receptors to those whose effect are primarily due to the release of endogenous transmitter (Nichols and Glennon, 1984). MDMA would, therefore, be a novel psychedelic by virtue of its effect on endogenous 5HT release rather than any activity at 5HT receptors. However, in previous studies on the release of tritiated 5HT and DA from striatal slices both (+)-MDMA and (+)-MDA were similarly potent 5HT releasing agents (Schmidt et al., in press) although (+)-MDA is reportedly without hallucinogenic activity (Glennon, 1984). Thus the behavioral effects of MDMA are certainly mediated by a mechanism more complex than just 5HT release.

In the present study, all three racemic methylenedioxyamphetamine derivatives appeared identical as 5HT releasing agents varying only in their effects on [^{3}H]DA release. Increasing the size of the N-alkyl substituent reduced the ability of the agent to enhance the efflux of [^{3}H]DA from striatal slices. This may explain some of the qualitative differences in the intoxication caused by the three drugs. For example, high doses of MDA are associated with a number of psychotoxic side-effects similar to those found with amphetamine abuse (Simpson and Rumack, 1981). Many of the central effects of amphetamine are believed to be a consequence the drug's potent effects on DA release (Groves and Rebec, 1976). Of the three methylenedioxyamphetamine derivatives, MDA was found to be the most potent at releasing DA. The less potent effects of MDMA and MDE on DA release may contribute to the milder form of intoxication described for these drugs and the lower incidence of amphetamine-like side-effects.

The complex neurochemical effects of this group of drugs makes it unlikely that any single mechanism will be found to be responsible for their behavioral effects. We have recently reported the existence of a binding site for [^{3}H]MDMA in rat brain which appears to be unique from a 5HT site (Gehlert et al., 1985). Further studies on this binding site are now in progress to determine if it may be involved in some of the unique behavioral properties of MDMA.

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