# 9. NEUROCHEMICAL EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE IN THE RAT: ACUTE VERSUS LONG-TERM CHANGES

CHRISTOPHER J. SCHMIDT AND VICKI L. TAYLOR

## INTRODUCTION

Amphetamine-like central stimulants are one of the most well-studied classes of pharmacological agents known today. This is due to the fact that their behavioral activity is believed to be mediated primarily by the monoamines, which themselves have been scrutinized sufficiently to have earned the title "classical transmitters." In spite of the attention given this class of agents and their relatively well-described neurochemical activities, there remains a great deal about these drugs that we do not understand. The neurotoxicity associated with high doses of many of these agents is one of these unexplained actions. Although the term high dose is used, it is important to point out that these doses are often in the range of which humans are exposed. This is particularly true in the case of 3,4-methylenedioxymethamphetamine (MDMA), as has been described elsewhere in this book. This neurotoxicity of the amphetamines is selective, in that it primarily affects the neuronal systems through which the drugs mediate their behavioral effects, i.e., the monoaminergic systems. Thus amphetamine, which is believed to cause the majority of its stimulant activities through dopamine release, causes persistent damage selectively to dopaminergic processes [1]. Methamphetamine, which is also a potent releaser of 5-HT, is neurotoxic to both the dopaminergic and serotonergic systems [2]. Finally, the selective serotonergic neurotoxicity of p-chloroamphetamine (PCA) correlates with 5-HT release as the presumed basis of most, though not all, of its behavior effects [3]. This pattern and some of

*Peroutka S.J. (ed), Ecstasy.*

our own data (to be reviewed here) have suggested to us that transmitter release may also be involved, in some manner, in the long-term effects of these drugs. This hypothesis has to a large extent determined the focus of our research effort on MDMA.

Due to this empirical relationship between behavior, acute neurochemistry, and neurotoxicity, our studies of MDMA have dealt exclusively with the effects of single administration of the drug. This reflects the human abuse situation more accurately and allows us to discern the acute neurochemical changes produced by the drug, through the use of both in vitro and in vivo models. Not surprisingly, results from these studies indicate that MDMA is similar in a number of regards to previously studied amphetamines. In its overall spectrum of neurochemical effects, however, MDMA most closely resembles the serotonergic neurotoxin PCA.

## 2. NEUROCHEMICAL EFFECTS OF MDMA

The administration of MDMA to rats produces rapid and pronounced reductions in central concentrations of 5-HT. This depletion of transmitter concentrations is widespread and involves a majority of the terminal field of the ascending serotonergic system. In contrast, the effects of MDMA on the dopaminergic system consist of a modest increase in dopamine concentrations, which return to control in a few hours [4]. Figure 1A shows the changes for cortical, hippocampal, and striatal 5-HT three hours following a single injection of MDMA (10 mg/kg, s.c.). Interestingly, when 5-HT concentrations are measured 24 hours following MDMA administration, the only significant effects observed are in the striatum (Figure 1B), suggesting a complete recovery in the other two regions. Further examination revealed that MDMA has a complex pattern of effect on 5-HT concentration, which differed slightly between brain regions. As shown in Figure 2 for the cortex and striatum, MDMA produces two distinct phases of transmitter depletion in the rat brain. This is most obvious in the cortex, where the transient recovery of 5-HT concentrations reaches control levels before the second phase of depletion begins. Although a recovery phase does seem to be present in the striatum, 5-HT concentrations do not return completely to control levels [4].

A number of investigators [5–7] have reported similar biphasic changes in 5-HT concentrations following the administration of PCA to rats. Studies from these laboratories have revealed that the initial effects of PCA are due to reversible alterations in serotonin synthesis, uptake, and release, whereas the long-term effect of the drug on serotonergic neurons is neurotoxic, involving a selective degeneration of the nerve terminals. PCA apparently produces little or no neurochemical or histological damage at the level of the cell bodies [7–9]. Our own results indicate that these effects of PCA also occur in the case of MDMA. Examination of various parameters of serotonergic function after MDMA administrations confirmed that one of the first alterations occurs in the activity of the rate-limiting enzyme for 5-HT synthesis,

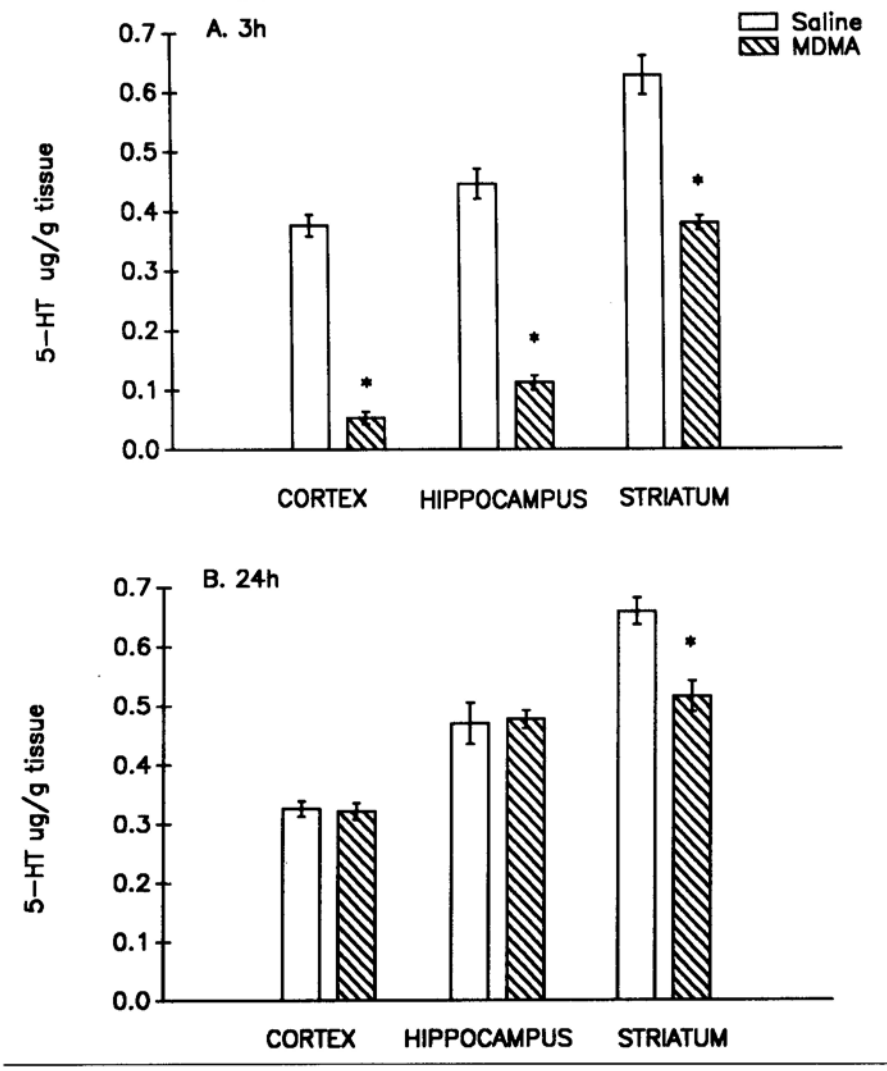


**Figure 1.** Effect of MDMA on regional brain concentrations of 5-HT in the rat at 3 (A) and 24 (B) hours after a single administration (10 mg/kg, s.c.). All error bars represent the SEM. *$P < 0.05$ versus saline.

tryptophan hydroxylase (TPH). This change actually precedes the decrease in 5-HT concentrations, as shown for the striatum in Figure 3. Similar results are observed in the cortex and the hippocampus, while the brainstem appears largely resistant to the effects of MDMA [10]. The data in Figure 3 also show that the decline in tryptophan hydroxylase activity begins as quickly as 15 minutes after drug administration. In most cases this is prior to any behavioral

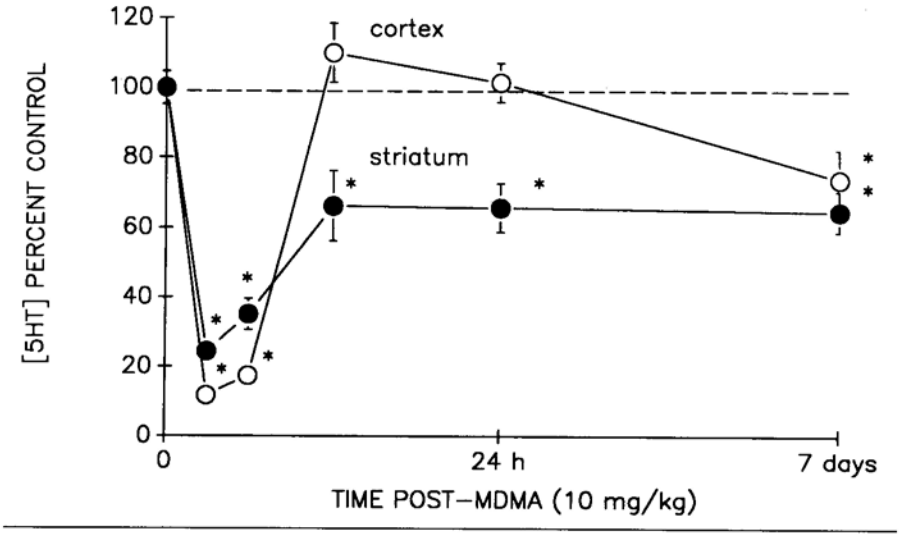


**Figure 2.** Time course of the changes in 5-HT concentrations in the cerebral cortex and striatum after a single 10 mg/kg dose of MDMA. *$P < 0.05$ versus control.

effect of the drug becoming apparent. A slight elevation in 5-HIAA at the earlier time points suggests that an increase in 5-HT turnover may be an initial event in these changes as well. This is consistent with results from in vitro release studies using striatal slices preloaded with [$^3$H]5-HT [11]. These experiments showed MDMA to be a potent 5-HT releasing agent, much like PCA. Collectively, these findings indicate that the first effects of MDMA on serotonergic neurons are a decrease in TPH activity and an increase in transmitter turnover. With TPH no longer acting to maintain transmitter levels, the releasing action of MDMA begins to deplete the nerve terminal of 5-HT. During this period (one to four hours) the animals exhibit most of the behavioral effects of MDMA. The recovery of 5-HT concentrations, shown in Figure 2, begins after most of the behavioral effects of MDMA have started to wane (four to six hours after drug administration), suggesting that the drug is no longer producing 5-HT release. Under these conditions, even the small 20% to 30% residum of TPH activity in the terminal can gradually restore levels of 5-HT to near control concentrations. Therefore, the acute effect of MDMA on 5-HT concentrations is due to both actions of the drug occurring more or less simultaneously at the nerve terminal. This requirement for both processes is demonstrated experimentally in the results shown in Figure 4. Administration of the 5-HT uptake inhibitor citalopram, which blocks the carrier-mediated release of transmitter [11], completely prevents the depletion of 5-HT measured at three hours after MDMA, in spite of tryptophan hydroxylase activity still being depressed 50% by MDMA. While these results

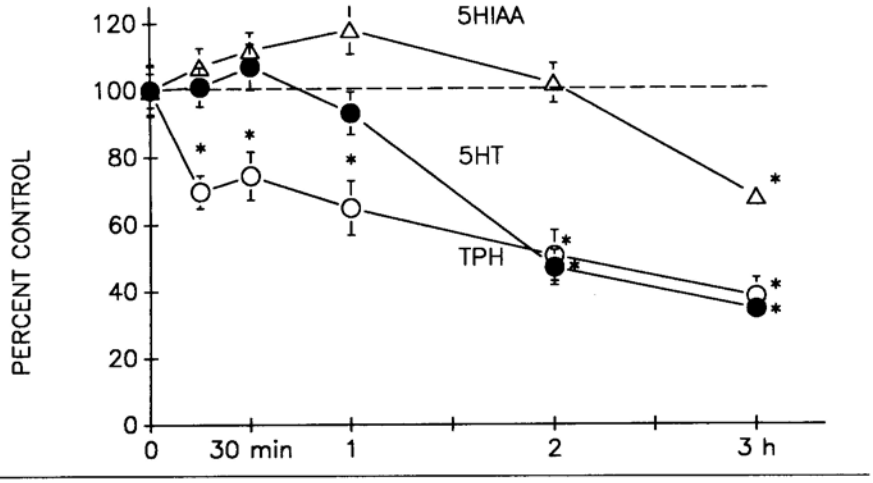


**Figure 3.** Acute time course of the effects of 10 mg/kg MDMA on serotonergic parameters in the striatum. *$P < 0.05$ versus control.

identify the processes operating at the level of the nerve terminal that lead to the gross neurochemical changes, the mechanism(s) that ultimately initiates these processes remains to be determined.

As part of our initial efforts to identify this mechanism(s), we began to characterize the acute and long-term effects of MDMA. Figure 5 displays results from an experiment examining the stereochemical requirements of each effect by comparing the optical isomers of MDMA for their ability to produce 5-HT depletion at three hours or at seven days. As shown in Figure 5A, either stereoisomer reduces striatal 5-HT concentrations acutely, with the 10 mg/kg dose already giving the maximum effect. In Figure 5B the results for the 20 mg/kg dose at seven days are plotted together with measurements of whole brain synaptosomal [$^3$H]5-HT uptake. At this time point, the (−)-stereoisomer of MDMA is without any significant effect on either parameter, while (+)-MDMA produces both a depletion in striatal 5-HT concentrations and a reduction in the uptake of [$^3$H]5-HT [11, 12]. Similar results were observed when cortical indoles were measured [12]. The reduction in the uptake of [$^3$H]5-HT has been demonstrated to be due to a decrease in the $V_{max}$ of the 5-HT transporter, without any alteration in its affinity for 5-HT as shown in Table 1 [12]. Consequently, these changes in transmitter uptake represent a loss of functional 5-HT uptake sites and provide biochemical evidence of neurotoxicity at the terminal level.

The results shown in Figure 5, therefore, indicate that the mechanisms leading to the acute and neurotoxic effects of MDMA have different stereochemical requirements or are somehow influenced differentially by the stereochemistry of the drug. An obvious explanation would be a difference in the

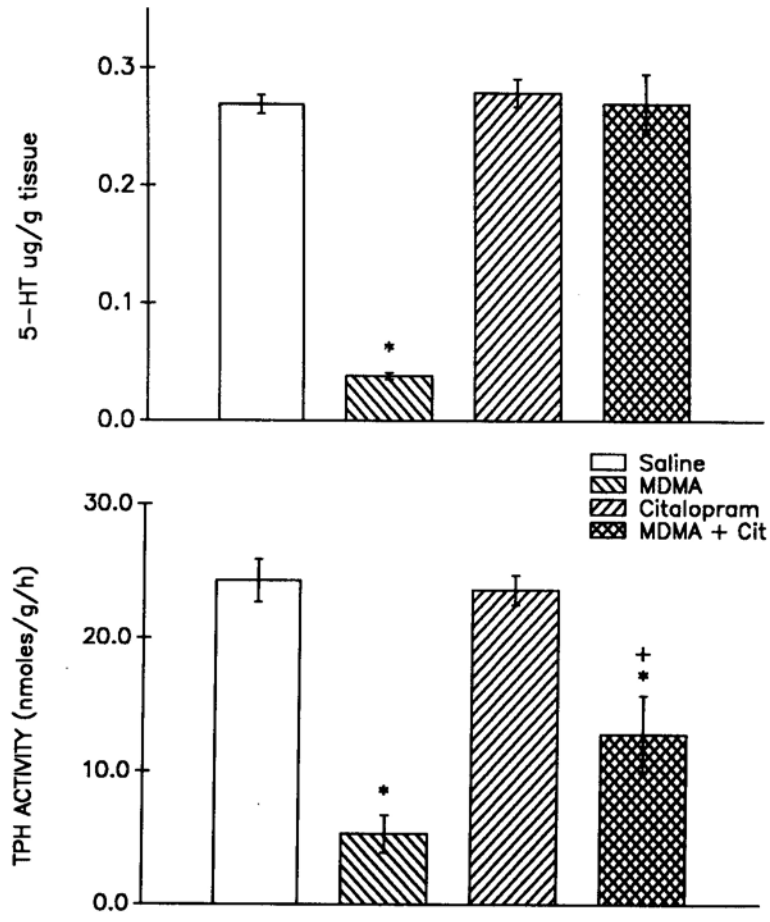


**Figure 4.** Effect of the simultaneous administration of the 5-HT uptake inhibitor citalopram on the acute reduction in cortical 5-HT concentrations (top) and cortical TPH activity (bottom) produced by 10 mg/kg MDMA at 3 hours. *$P < 0.05$ versus MDMA alone.

rate of metabolism of the two enantiomers, leading to a difference in their brain concentrations. However, we believe this divergence in the neurochemical effects of the two stereoisomers at one week involves more than differences in their rate of metabolism. This reasoning is based in part on comparison with PCA, which has been shown to have an identical stereochemical preference for the production of its acute, versus neurotoxic, effects in rats. When brain concentrations of (+)-PCA and (−)-PCA were measured, the half-lives of the two enantiomers were found to be identical; hence, a difference in the duration of drug action cannot account for the greater neurotoxicity of (+)-PCA compared to (−)-PCA [13]. Similar studies of the metabolism

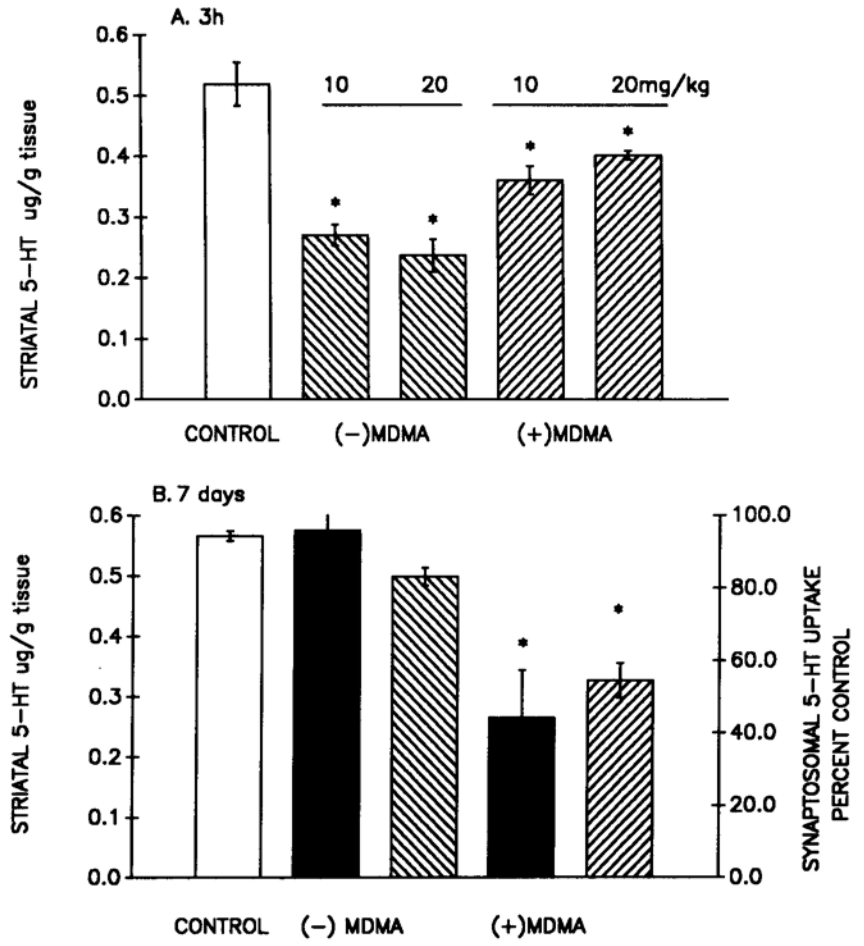


**Figure 5.** Comparison of the acute (A) and long-term effects (7 days) of the stereoisomers of MDMA. Data for both 10 and 20 mg/kg are shown for the 3 hour time point while the effects of the higher dose are shown in the lower figure. Synaptosomal uptake of [$^3$H]5-HT (black bars) was measured in whole brain synaptosomes. *$P < 0.05$ versus control.

of MDMA and its stereoisomers are required to determine if variations in the rate of metabolism could account for the differences in the long-term effects of the two enantiomers.

Interestingly, the effect of (+)-MDMA and (−)-MDMA on neurotransmitter release in vitro shows a correlation with the apparent difference in the stereochemical stringency observed for the acute and neurotoxic effects of MDMA in vivo. Whereas there was little difference between the stereoisomers in terms of their effect on [$^3$H]5-HT release, (+)-MDMA was a considerably more potent releasing agent for [$^3$H]-dopamine than was (−)-MDMA [11].

**Table 1.** Kinetic parameters for the uptake of [$^3$H]5-HT by rat $P_2$ synaptosomes 7 days following (+)-MDMA (20 mg/kg) or saline administration. *$P < 0.05$ versus control.

| | | Uptake | |
|---|---|---|---|
| | Cortical 5-HT (μg/g) | $V_{max}$ (dpm/mg/5 min) | $K_m$ (μM) |
| Control | 0.39 ± 0.03 | 105080 ± 6568 | 0.037 ± 0.003 |
| MDMA | 0.21 ± 0.02* | 73546 ± 10720* | 0.044 ± 0.004 |

Similar results were observed by Johnson et al. [14] and Steele et al. [15].

The existence of several homologues of MDMA provides another strategy for examining the relationship between MDMA's acute and neurotoxic effects. Table 2 contains data comparing the acute and long-term effects of MDMA with those of its desmethyl and N-ethyl analogues, methylenedioxyamphetamine (MDA) and N-ethyl-methylenedioxyamphetamine (MDE), respectively. At the single dose of 20 mg/kg used, all three drugs produced massive depletions of cortical 5-HT at the three hour time point. However, at seven days the same dose of the compounds produced depletions only in animals administered MDA or MDMA. These drugs also produced the decrease in the uptake of [$^3$H]5-HT by whole brain synaptosomes, indicative of neurotoxicity [16]. These data are thus similar to the results observed for the stereoisomers of MDMA, in that the drugs appeared similar at the three hours time point, with significant differences only becoming apparent one week later. The lack of any residual effect of MDE, and (−)-MDMA in the previously discussed experiment, proves the effect of these drugs on tryptophan hydroxylase activity is reversible in as little as one week and is not due to a neurotoxic response at the nerve terminal. This further suggests that different mechanisms are responsible for the production of the two effects. The divergence in the neurochemical response to the three homologues after one week could be attributed to differences in their rates of metabolism, as already discussed for the stereoisomers of MDMA. Even if this is the case, the results indicate at least a pharmacokinetic difference exists in the requirements for the production of the acute effect, versus the neurotoxicity. Metabolic studies are required to determine if such a difference in the disposition of these drugs does exist.

The corresponding in vitro release experiments with the three homologues also yielded results similar to the experiments with the enantiomers of MDMA. All three drugs were found to be essentially identical in terms of their potency for producing [$^3$H]5-HT release. When their effects on [$^3$H]-dopamine release were determined, however, the three drugs were significantly different, with a rank order of potency of MDA > MDMA > MDE [16]. Thus, [$^3$H]-dopamine release follows the same rank order as does he neurotoxicity of the three drugs.

The rapid onset and rate of decrease in TPH activity following the adminis-

tration of MDMA or its homologues to rats are among the most dramatic neurochemical effects produced by any class of CNS active agents. This phenomenon also occurs acutely after the administration of PCA [5, 6], methamphetamine [17, 18], or fenfluramine [19]. The reversibility of this effect indicates it is not due to damage to the integrity of the serotonergic nerve terminal. Since all these agents release large quantities of 5-HT from the terminal, it seems entirely possible that a decrease in the activity of TPH might merely be a homeostatic response on the part of the neuron. To determine if an allosteric modification of the enzyme is responsible for the loss of TPH activity following MDMA administration, we compared the kinetic characteristics of the enzyme in cortical homogenates from animals treated three hours previously with MDMA to that of saline-treated animals. Figure 6 shows there was no change in the affinity of the enzyme for either its substrate, tryptophan, or the synthetic cofactor, 6-methyl-tetrahydropterine, after MDMA administration. There was however a significant decrease in the $V_{max}$ activity of the enzyme, in this case amounting to approximately 50% [20]. We have shown this is not due to a direct effect of MDMA on the enzyme in vitro [20], and experiments combining cortical homogenates from saline- and MDMA-treated rats failed to yield any suggestion of a metabolite that might directly affect TPH activity [Schmidt and Taylor, unpublished results]. These data and the $V_{max}$ decrease in enzyme activity suggest a complete inactivation of tryptophan hydroxylase, as does the lack of a recovery phase during the same period as the transient recovery of 5-HT concentrations. Complete recovery of TPH activity may depend upon synthesis of a new enzyme in the cell bodies, with subsequent transport from the brainstem to the terminals. Assuming that this process requires several days, replenishment of the terminals with enzyme would eventually be blocked by the MDMA-induced degeneration of the nerve terminals. In the case of (−)-MDMA and MDE, where no long-term or neurodegenerative effects develop, complete recovery of TPH activity and, hence, transmitter concentrations could occur.

We have been unable to show a MDMA-induced loss of tryptophan hydroxylase activity using either synaptosomes or superfused slices of cerebral cortex exposed to high concentrations of the drug [10], although it is apparent that MDMA does release 5-HT from such preparations [21]. This suggests that an intact neuronal network is necessary for this effect or that in vivo metabolism of the drug is required. To address the first possibility, we made direct injections of MDMA stereotaxically into the brains of rats under metophane anesthetic. Three injection sites were selected, with each group having their own saline injected controls. All animals were allowed to survive for three hours after injection, to observe any behavioral effecs of the drug. TPH activity as well as 5-HT concentrations were determined in a number of regions for each injection site. Results from the assay of cortical TPH activity are shown in Figure 7. The injection of 300 μg of MDMA directly into the

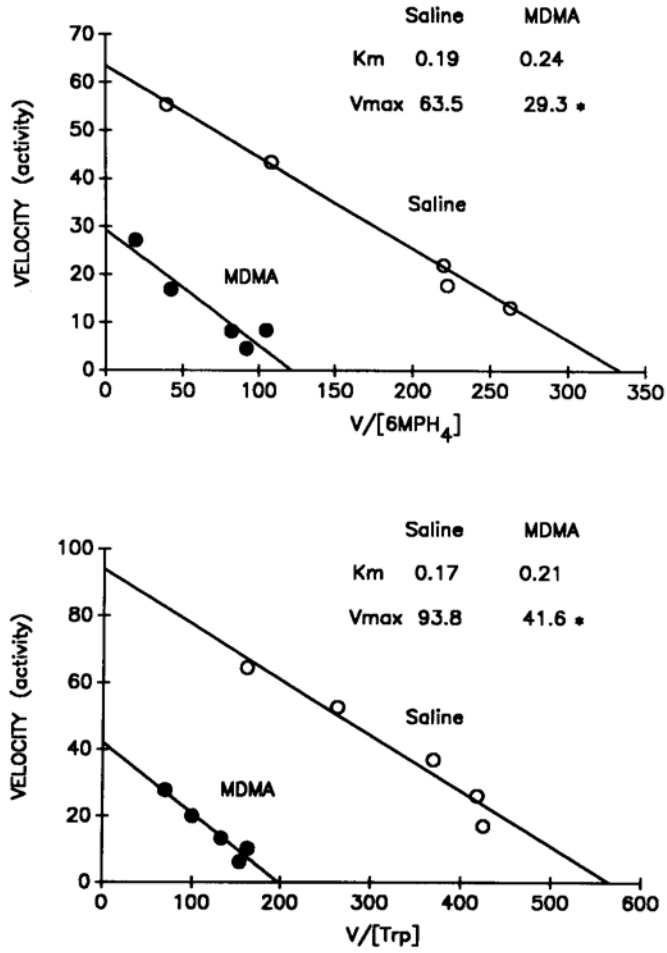


**Figure 6.** Effect of the acute MDMA (10 mg/kg) on the kinetics of cortical TPH at 3 hours with respect to $6MPH_4$ (top) or tryptophan (bottom). The data are shown as Eadie–Hofstee plots. Values for the $K_m$ and $V_{max}$ of the enzyme for control and MDMA-treated rats are provided in the figure. $^*P < 0.005$ versus control.

substantia nigra, the dorsal raphe, or the cerebral ventricles had no effect on cortical TPH activity. Similar results were observed for TPH activity and 5-HT concentrations in the striatum and hippocampus, regardless of the injection site [10]. Repeating the i.c.v. injections using pentobarbital as the anesthetic did not alter the outcome of the experiment. Hence, direct application of MDMA to either the terminal field or cell bodies of serotonergic neurons did not reproduce the acute effects of peripheral administration. In addition to the lack of neurochemical effects, these injections produced no

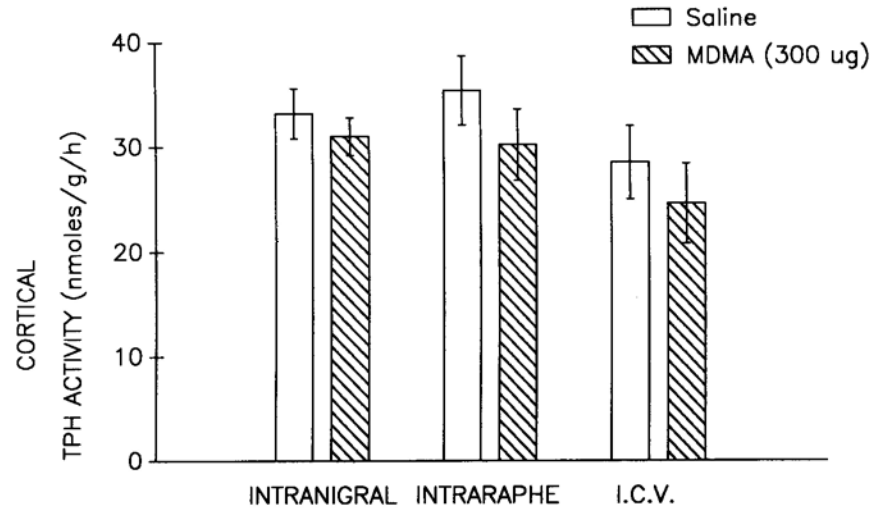


**Figure 7.** Lack of effect of direct injections of MDMA (300 μg) into various brain sites on the activity of TPH in the cerebral cortex. The drug was stereotaxically injected under a light metophane anesthetic and the animals were sacrificed 3 hours later. Similar results were observed for 5-HT concentrations and in all other areas examined.

obvious behavioral effects in the animals, with the exception of some contralateral turning in the nigra-injected rats. This led us to question the validity of using local injections of a small quantity of a lipophilic drug such as MDMA as a model for determing its central actions. Using [$^3$H]MDA, Marquardt et al. [22] showed that 10% of a peripherally administered dose of the drug was present in the brain within 30 minutes of injection. For a 300 g rat given 10 mg/kg, this would amount to the 300 μg of drug we used in our studies. Since the administration of this much compound did not affect any of the neurochemical parameters measured, it is likely the drug rapidly distributed throughout the animal at a concentration too low to have any effect. Consequently, we elected to use direct i.c.v. infusions of MDMA into conscious animals to insure that behaviorally relevant brain concentrations of the drug were maintained for a period of time similar to that which might be expected following peripheral administration. Using this approach, we have been able to demonstrate significant reductions in regional tryptophan hydroxylase activity with infused doses of MDMA as low as 300 μg or a total body dose of approximately 1 mg/kg [10]; these data are shown for the cerebral cortex in Figure 8. The absence of any change in 5-HT concentrations while enzyme activity is significantly decreased is interesting in light of our observation that the loss of enzyme activity precedes the decline in transmitter concentrations. Since higher infusion doses, i.e., 600 μg, reduced both TPH activity and 5-HT concentration, the 300 μg dose may have been sufficient to elicit the loss

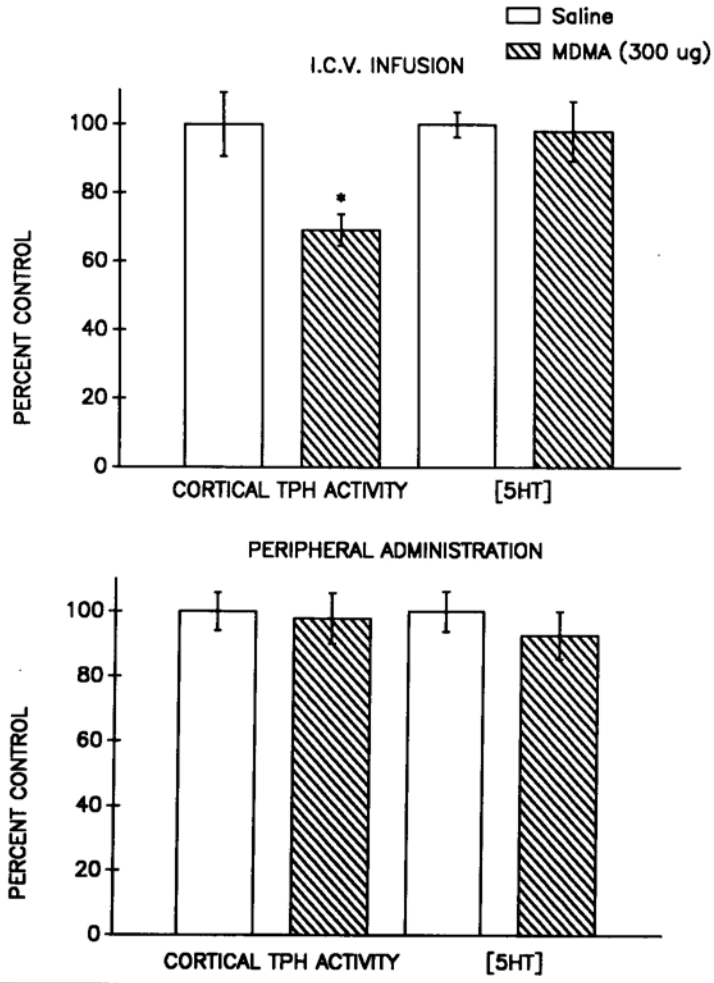


**Figure 8.** Comparison of the effect of a continuous i.c.v. infusion of MDMA with peripheral administration of the same dose. MDMA was infused through a stainless steel cannula for 1 hour, after which the rats were observed for an additional 2 hours prior to sacrifice. MDMA was administered peripherally by the s.c. route and the animals were sacrificed 3 hours later. $^*P < 0.05$ versus saline.

of enzyme activity without producing enough release to actually deplete the terminals. More important is the point that 300 μg is the same dose that had no effect when given by a bolus injection centrally, nor did it affect enzyme activity following peripheral administration, as also shown in Figure 8. These data, therefore, demonstrate that the acute effect of MDMA on serotonergic neurons is a central action of the drug, but one requiring sustained, high concentrations of the agent.

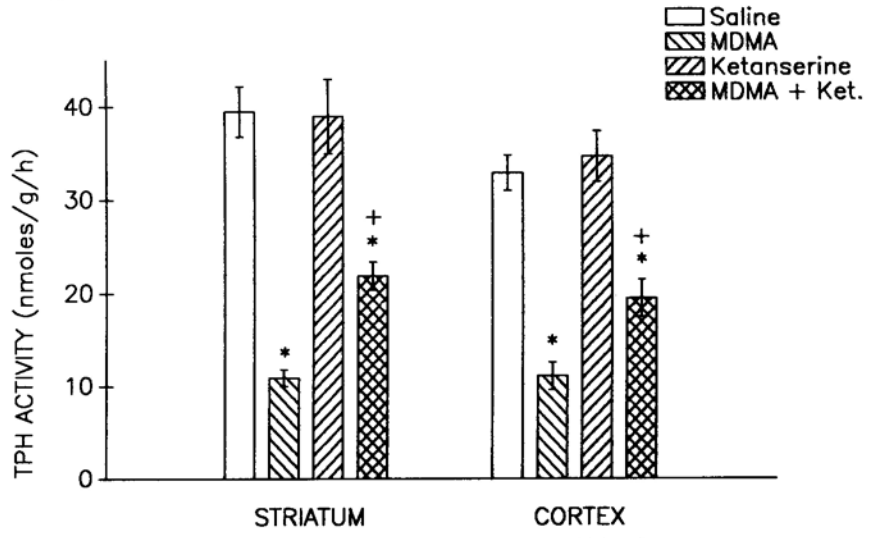


**Figure 9.** Partial antagonism of the acute loss of striatal and cortical TPH activity by the 5-$HT_2$ receptor antagonist ketanserine. MDMA (10 mg/kg) and ketanserine (2.5 mg/kg) were administered simultaneously 3 hours prior to sacrifice. *$P < 0.05$ versus saline; +$P < 0.05$ versus MDMA alone.

Although we believe we have excluded the possible involvement of a peripheral metabolite of MDMA in the acute effect of the drug on serotonergic neurons, the mechanism ultimately responsible for the loss of TPH activity has remained elusive. We have considered a number of possible mechanisms that would be a consequence of the release of monoaminergic transmitters produced by MDMA. Interruption of catecholamine biosynthesis by pretreatment with α-methyl-p-trysoine had no consistent effect on the loss TPH activity or the acute depletion of 5-HT produced by MDMA. Unilateral lesions of the substantia nigra with 6-hydroxydopamine were similarly without effects [Schmidt and Taylor, unpublished results]. More global depletions of monoamine stores, with reserpine [20] or the L. aromatic-amino acid decarboxylase inhibitor, α-fluoromethyldopa, also failed to block loss of TPH activity produced by MDMA [Schmidt and Taylor, unpublished, results]. With the exception of inhibitors of the 5-HT uptake carrier system, the only pharmacological manipulations that have consistently altered the acute effect of MDMA have been a modest antagonism with 5-HT receptor antagonists, such as ketanserine. The data for cortical and striatal TPH activity are shown in Figure 9. Similar results were observed with the nonselective 5-HT antagonist methiothepin [20]. Neither antagonist had any significant effect on the decrease in 5-HT concentrations after MDMA administration, however. This is not surprising in view of the very small protection provided by the drugs and the fact that receptor antagonists would not alter the carrier-mediated

**Table 2.** Comparison of the acute and long-term effects of MDMA and its analgoues on serotonergic neurons in the rat.

| | Cortical 5-HT μ/g tissue | | [$^3$H]5-HT uptake at 7 days |
|---|---|---|---|
| | 3 hours | 7 days | % control |
| Saline | 0.323 ± 0.016 | 0.280 ± 0.017 | 100 ± 5.1 |
| MDA (20 mg/kg) | 0.056 ± 0.006* | 0.093 ± 0.015* | 61.2 ± 8.2* |
| MDMA (20 mg/kg) | 0.036 ± 0.004* | 0.124 ± 0.020* | 64.0 ± 1.7* |
| MDE (20 mg/kg) | 0.052 ± 0.004* | 0.297 ± 0.025 | 99.6 ± 3.5 |

efflux of 5-HT from the nerve terminals. Unfortunately, the effect of the antagonists is small, and further work with other 5-HT receptor blockers is required to determine if excessive activity at 5-HT receptors is somehow involved in the acute effects of MDMA on TPH.

Although the reversibility of the acute effect of MDMA is well demonstrated by the results with the (−)-stereoisomer of MDMA (see Figure 5) and the results with MDE (see Table 2), the difference between the development of the acute and long-term neurochemical effects of MDMA is most clearly shown by the results displayed in Figure 10. In this experiment, rats were administered MDMA at time zero, with the 5-HT uptake inhibitor, fluoxetine, being administered either simultaneously or at various times after MDMA. All animals were sacrified at one week. Both cortical TPH activity and 5-HT concentrations are represented in the figure as a percent of the appropriate control group: either fluoxetine or saline at each time point. Simultaneous administration of fluoxetine with MDMA completely blocks the long-term depletion of 5-HT indicative of neurotoxicity, demonstrating that both the acute and long-term depletion of 5-HT by MDMA are sensitive to inhibitors of 5-HT uptake. Three hours after MDMA administration, 5-HT concentrations are fully depressed due to the acute effects of MDMA, yet administration of the uptake inhibitor at this time still prevents development of the neurotoxicity. Fluoxetine at six hours after MDMA provided partial protection but was without effect by 12 hours post-MDMA [12]. Similar results were observed for cortical TPH activity, although inhibition of uptake did not appear to block the loss of enzyme activity beyond three hours post-MDMA. This smaller window for protection of TPH activity may be due to the quicker response of the enzyme to MDMA, when compared to 5-HT concentrations (see Figure 3). The observation that tryptophan hydroxylase activity does recover by one week when fluoxetine is administered at three hours is further support for the hypothesis that synthesis of new enzyme is required to restore enzyme activity on the affected nerve terminals.

The ability to block the development of the neurotoxicity after the serotonergic terminal has been depleted of 5-HT suggests the massive MDMA-induced release of 5-HT is not responsible for the long-term effects of the drug. However, the results show that, in addition to the role of the 5-HT

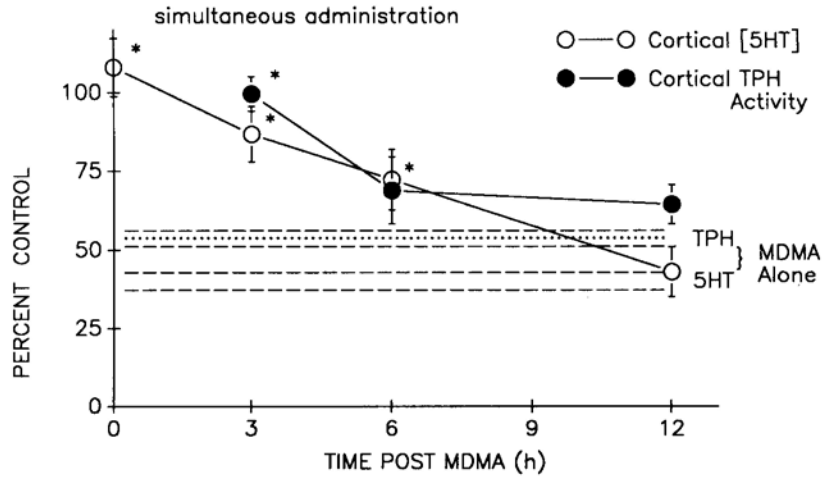


**Figure 10.** Time-dependency of the blockade of MDMA-induced neurotoxicity by fluoxetine. Animals were injected with MDMA (20 mg/kg) at time zero, followed by injection of saline or fluoxetin (5 mg/kg) at various times thereafter. All rats were sacrificed 1 week later. The effect of MDMA alone on TPH activity and 5-HT concentrations is represented by the horizontal bars. *$P < 0.05$ versus MDMA alone.

carrier in the acute depletion of 5-HT by MDMA, some late activity on the part of the carrier must be required for the development of the neurotoxic response to MDMA. By three hours the serotonergic terminal has been depleted of 5-HT; hence, fluoxetine can no longer be interfering with this activity, and by six hours, most of the behavioral effects of MDMA have abated. However, even this late interference with the activity of the uptake carrier can disrupt the development of the neurotoxicity. Because this late activity on the part of the carrier is occurring between three and 12 hours after MDMA, it is possible that a metabolite of the drug is being accumulated during this period. A similar hypothesis has been offered to explain the neurotoxicity of PCA and its sensitivity to fluoxetine for as long as 48 hours after drug administration [5]. Although MDMA is less potent than PCA as a neurotoxin, its effect apparently evolves in a shorter time period since it cannot be blocked beyond six hours after MDMA administration.

Although the generation of a hypothetical neurotoxic metabolite of MDMA is compatible with the apparent stereochemistry of the neurotoxic effect, by analogy with PCA, there are a number of problems with this hypothesis. Similar stereochemical specificity for the neurotoxic effect of PCA has reinforced the opinion that a neurotoxic metabolite of PCA may be involved in its long-term effects on serotonergic neurons. However, although there have been reports of covalent binding of a metabolite of PCA to cell macromolecules in vitro, [23, 24], this metabolite has yet to be isolated. The neurotoxi-

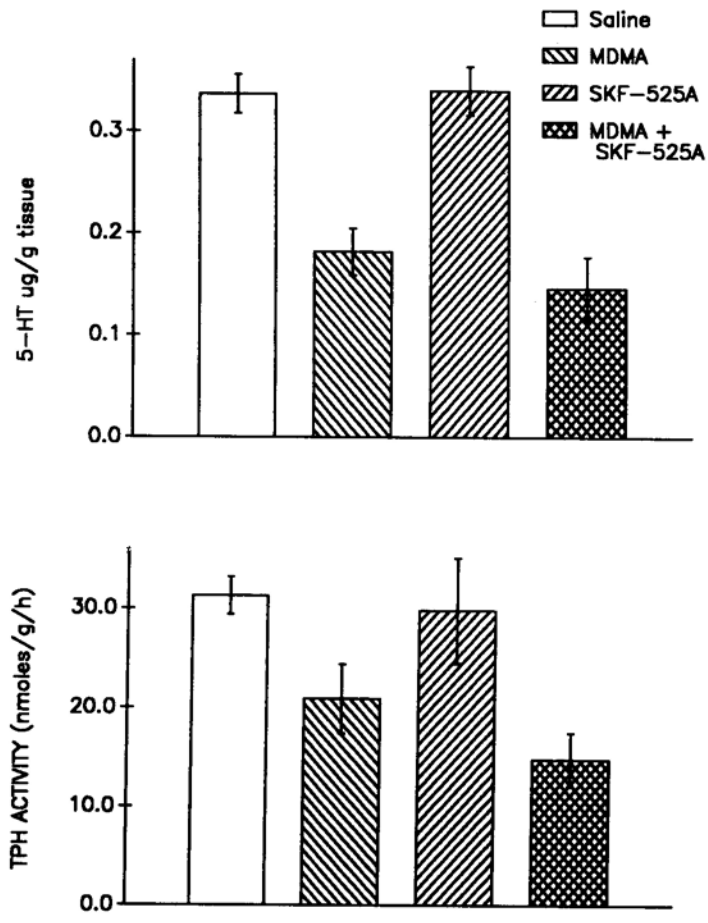


**Figure 11.** Lack of effect of the cytochrome P-450 inhibitor SKF-525A on the neurotoxicity of MDMA. SKF-525A (10 mg/kg, i.p.) was given 60 minutes prior to MDMA (20 mg/kg) and all rats were sacrificed 1 week later.

city of PCA is also unaffected by intracerebral glutathione [25] or pretreatment with the cytochrome P-450 inhibitor, SKF-525A [26]. Studies by Steranka and Sanders–Bush [27], using metabolic inducers such as phenobarbital and 3-methylcholanthrene, failed to find evidence of a neurotoxic hepatic metabolite of PCA. We have treated rats with SKF-525A prior to MDMA administration without any effect on either the long-term loss of TPH activity or 5-HT concentrations (see Figure 11).

In the absence of more evidence for a neurotoxic metabolite of MDMA or PCA, we are considering other possible mechanisms for the long-term effects of these drugs. These include the generation of a neurotoxic molecule from

one or more of the transmitters released by these drugs, as well as direct receptor effects of the released transmitters. There is considerable evidence suggesting a role for dopamine release in the neurotoxicity of methamphetamine, including sensitivity to pretreatment with α-methly-p-tyrosine [28, 29] and 6-hydroxydopamine lesions of the substania nigra [30]. Dopamine receptor antagonists have also been found to alter methamphetamine-induced neurotoxicity [31]. Similar studies are currently under way in our laboratory, with agents selected to interfere with the function of either serotonergic or catecholaminergic systems.

In conclusion, studies in our laboratory have identified two distinct neurochemical consequences of MDMA administration to rats. The initial effects of MDMA on the serotonergic nerve terminal include an uregulated carrier-mediated release of 5-HT coupled with a rapid loss of tryptophan hydroxylase activity. Both of these events are necessary for the massive depletion of forebrain 5-HT concentrations that occur after the administration of MDMA. The loss of enzyme activity is a centrally-mediated effect of the drug, although the ultimate mechanism remains to be determined. Although tryptophan hydroxylase is apparently irreversibly inactivated, the acute effect of MDMA on the neuron is not an irrevocable event that commits the nerve terminal to degeneration, since (−)-MDMA or MDA can both produce a depletion of 5-HT quantitatively similar to (+)-MDMA or MDA, without producing evidence of neurotoxicity at later times. The ability of fluoxetine to block the process leading to the neurotoxicity after the acute depletion of 5-HT has already occurred also supports the reversibility of these early changes. In all respects, the neurochemical effects of MDMA qualitatively resemble those of PCA, suggesting the drugs may also share a common mechanism of action. This is likely to be true with regard to both their acute and neurotoxic effects. Although current opinion favors neurotoxic metabolite as the cause of the long-term effects of PCA, there is as yet no conclusive data to validate this hypothesis. MDMA has not been subject to the same degree of investigation as has PCA, however, evidence for its metabolism to a neurotoxin is also scant. In the absence of such data, we suggest that the evidence of a role for neurotransmitter release in the neurotoxicity of amphetamines is at least as strong as that for a neurotoxic metabolic. There is good evidence that dopamine release is a prerequisite for the neurotoxic effects of methamphetamine on both dopaminergic and serotonergic neurons. In the case of MDMA, the stereochemical specificity and structural requirements for the production of the neurotoxicity are also compatible with the requirements for dopamine release.

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