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5-HT_2 Antagonists Stereoselectively Prevent the Neurotoxicity of 3,4-Methylenedioxymethamphetamine by Blocking the Acute Stimulation of Dopamine Synthesis: Reversal by L-Dopa

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ABSTRACT

The active and inactive stereoisomers of the serotonin (5-HT_2) antagonist, MDL 11,939, were used to examine the relationship between the acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on the dopaminergic system and its long-term effects on the serotonergic system. Only the R-(+) stereoisomer of MDL 11,939 both reversed the acute stimulation of striatal dopamine synthesis by MDMA and prevented the deficit in forebrain 5-HT concentrations measured one week later. This acute activation of striatal dopamine synthesis by MDMA is a compensatory response to the carrier-mediated efflux of transmitter as shown by its sensitivity to the dopamine uptake inhibitor, nomifensine. It is suggested that in the absence of this enhanced synthesis, the dopaminergic neuron cannot sustain the carrier-mediated dopamine release which is a prerequisite for the development of MDMA-induced neurotoxicity. This hypothesis is supported by the observation that the administration of the dopamine precursor, L-dopa, with MDMA reverses the protective effects of 5-HT_2 receptor antagonists.

MDMA is currently a popular drug of abuse due to its unique combination of both euphoric and stimulant properties (Siegel, 1986; Peroutka *et al.*, 1988). In addition to its novel behavioral effects, the unusual neurochemical profile of MDMA has made it the subject of a number of recent investigations. Neurochemically, the drug has two temporally distinct effects on the serotonergic nervous system. Administration of a single dose of MDMA to rats initially causes a rapid and extensive depletion of 5-HT in the forebrain due to an increase in transmitter efflux coupled with a decline in the activity of tryptophan hydroxylase (Stone *et al.*, 1986; Schmidt, 1987; Schmidt and Taylor, 1987). This acute effect of MDMA reaches its maximum 3 to 6 h after drug administration followed by a gradual recovery of transmitter concentrations as the releasing action of the drug wanes. Within one week of drug administration, however, a second and more persistent phase of 5-HT depletion develops. Both biochemical (Schmidt, 1987; Battaglia *et al.*, 1987; O'Hearn *et al.*, 1988) and histochemical (Commins *et al.*, 1987; Scallet *et al.*, 1988) markers have shown that this later decrease in transmitter concentrations is associated with damage to the serotonergic nerve terminal. This neurotoxic effect of MDMA has lead to serious concern over the potential hazards of human exposure to the drug (Peroutka, 1987; Price *et al.*, 1989; Edwards, 1989).

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Several studies have now provided data implicating dopamine release in the etiology of MDMA-induced neurotoxicity (Stone *et al.*, 1988; Schmidt *et al.*, 1990a). More recent reports showing that 5-HT_2 receptor antagonists block the long-term neurochemical changes produced by MDMA have also implicated 5-HT release and subsequent stimulation of 5-HT_2 receptors in this response. There are data available that suggest that MDMA-induced dopamine release and the release of 5-HT may be related. Nash *et al.* (1990) first reported that MDMA stimulated the accumulation of striatal dihydroxyphenylalanine in NSD 1015-treated rats *via* a mechanism sensitive to the 5-HT_2 receptor antagonist, ketanserin. In our own studies, we found that the acute increase in striatal dopamine concentrations observed after MDMA administration was sensitive to 5-HT_2 antagonists such as ritanserin and MDL 11,939 (Schmidt *et al.*, 1990b). Both observations suggest that 5-HT_2 receptors play a role in the stimulation of dopamine synthesis produced by MDMA. Furthermore, MDMA-induced changes in dopamine metabolites in the presence of a 5-HT_2 antagonist indicated that interference with dopamine synthesis by the 5-HT_2 antagonist may compromise MDMA-induced dopamine release.

In this paper, we examine the hypothesis that 5-HT_2 receptor antagonists block the neurotoxicity of MDMA by interfering with its acute stimulation of striatal dopamine synthesis. The basis of the MDMA-induced increase in striatal dopamine concentrations is characterized further and the role of these

ABBREVIATIONS: DOPAC, dihydroxyphenylacetic acid; MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine (serotonin); HVA, homovanillic acid.

acute changes in the long-term effects of MDMA is examined using the recently resolved stereoisomers of the selective 5-HT$_2$ antagonist, MDL 11,939 (Dudley *et al.*, 1988).

Materials and Methods

Male, Spraque-Dawley rats (250–300 g) were maintained on a fixed 12-h light-dark cycle and given food and water *ad libitum*. Acute experiments were performed using a 3-h time point whereas studies on the long-term effects of MDMA were terminated at one week. All animals were sacrificed by decapitation and their brains were rapidly removed and placed on ice. The appropriate regions were immediately dissected free, frozen on dry ice and stored at −70°C until assayed. All drugs were administered in saline by s.c. injection with the exception of carbidopa and L-dopa, which were administered i.p.

Tissue concentrations of dopamine, serotonin and their metabolites were determined by high performance liquid chromatography with electrochemical detection as described previously (Schmidt *et al.*, 1987).

Ritanserin and carbidopa were the kind gifts of Janssen Pharmaceutica (Beerse, Belgium) and Merck, Sharp and Dohme Research Laboratories (Rahway, NJ), respectively. Haloperidol was purchased from Research Biochemicals (Natrick, MA). L-dopa was purchased from Sigma Chemical Co. (St. Louis, MO). (±) MDL 11,939 was synthesized and stereochemically resolved at the Merrell Dow Research Institute (Cincinnati, OH). Stereochemical purity of the R-(+) enantiomer was 97.6%, whereas that of the S-(−) stereoisomer was 98.3% as determined by NMR (Nieduzak and Carr, 1990). MDMA and its stereoisomers were provided by the National Institute on Drug Abuse. All drugs with the exception of MDMA were given as the salt.

Results

Ligand binding studies have shown that the antagonist activity of MDL 11,939 at the 5-HT$_2$ receptor is due exclusively to the R-(+) stereoisomer (Dudley *et al.*, in preparation). The first series of experiments utilized the enantiomers of MDL 11,939 to determine if the 5-HT$_2$ antagonist stereoselectively blocked both the acute effect of MDMA on the dopaminergic system and its long-term effects on the serotonergic system. Figure 1 shows results from an experiment in which rats were sacrificed 3 h after the administration of MDMA (20 mg/kg s.c.) alone or in combination with (±)MDL 11,939 or one of its stereoisomers (5 mg/kg s.c.). The results are presented as the change in striatal concentrations of dopamine, DOPAC or HVA, respectively. Neither MDL 11,939 nor its isomers altered the acute depression of striatal 5-HT and 5-hydroxyindoleacetic acid concentrations produced by MDMA (not shown). As shown in the figure, dopamine concentrations were not altered after the administration of (±)MDL 11,939 nor either of its stereoisomers alone. However, as demonstrated previously, racemic MDL 11,939 completely blocked the MDMA-induced elevation of dopamine concentrations by converting a 32% increase in the absence of the antagonist to a 7% decrease in its presence ($P < .01$). Although both enantiomers of the antagonist attenuated the MDMA-induced elevation of dopamine concentrations, R-(+)MDL 11,939 produced a significantly greater effect than the S-(−) stereoisomer ($P < .05$). When given with MDMA, S-(−)MDL 11,939 returned dopamine levels to 106% of control whereas the R-(+) stereoisomer actually reduced dopamine levels to 73% of control. This decline in dopamine concentrations in the presence of the R-(+) enantiomer was statistically significant when compared to the saline controls ($P < .05$).

The administration of racemic MDL 11,939 significantly potentiated the MDMA-induced depression of striatal DOPAC

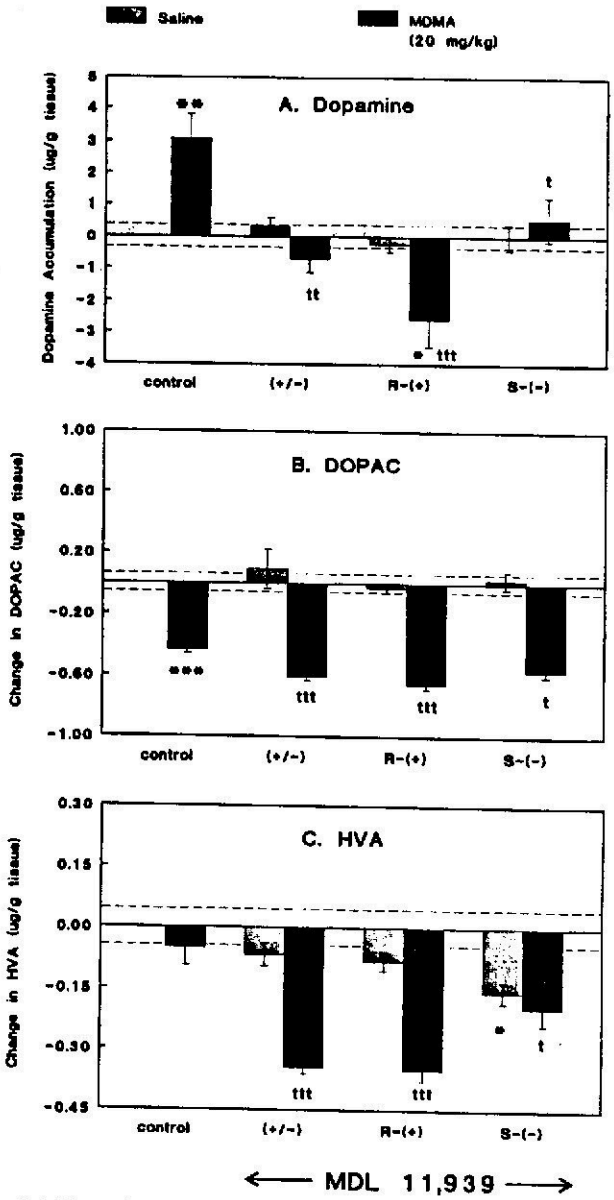

Fig. 1. Effect of MDL 11,939 and its stereoisomers on the acute alterations in striatal dopamine metabolism produced by MDMA. MDMA (20 mg/kg s.c.) and the antagonists (5 mg/kg s.c.) were administered simultaneously and the rats were sacrificed 3 h later. Results are presented as the mean change from the saline control ± the S.E.M. with $n = 5$ for each group. Control values were 9753 ± 361, 1010 ± 59, and 737 ± 45 ng/g for dopamine, DOPAC and HVA, respectively. *, **, *** = $P < .05, .01, .001$, respectively, *vs.* saline; t, tt, ttt = $P < .05, .01, .001$, respectively, *vs.* MDMA.

($P < .001$). Although there was no statistically significant difference in the activity of the two stereoisomers in this regard, a quantitatively greater effect was observed with the R-(+) enantiomer.

MDMA alone did not produce any alteration in the concentration of striatal HVA in this experiment. In the presence of MDL 11,939 however, MDMA produced a significant decrease in concentrations of this metabolite. R-(+)MDL 11,939 produced an effect identical to that of the racemate which was significantly greater than that of the S-(−) enantiomer. When compared directly to controls receiving only the S-(−) enantiomer, coadministration of MDMA plus the S-(−) stereoisomer had no effect on the concentration of HVA.

In another experiment, rats treated as described above were sacrificed 1 week after drug administration to determine if the stereoselectivity of the acute interaction between MDMA and MDL 11,939 was also observed for protection against MDMA-induced neurotoxicity. The administration of MDMA alone reduced cortical and hippocampal 5-HT concentrations to 57% and 64% of control, respectively (fig. 2). (±)MDL 11,939 antagonized this effect in both brain regions as shown previously. However, of the two enantiomers, only R-(+)MDL 11,939 produced a statistically significant reversal of the long-term effect of MDMA.

The basis of the acute effects of MDMA on the concentrations of striatal dopamine and its metabolites was studied in several experiments. Table 1 shows the effects of coadministration of the D2 antagonist haloperidol (2mg/kg s.c.) or the selective dopamine uptake blocker nomifensine (10 mg/kg s.c.) on the changes measured 3 h after MDMA administration. Haloperidol had no effect on the elevation of striatal dopamine by MDMA and in spite of producing significant elevations in both DOPAC and HVA alone, it did not significantly alter the effect of MDMA on either metabolite.

In contrast to the lack of any effect with haloperidol, nomifensine completely prevented the MDMA-induced increase in dopamine concentrations ($P < .01$) while not altering the depression of DOPAC. An increase in HVA concentrations with nomifensine was not appreciably altered by MDMA.

Table 2 shows data comparing the acute neurochemical effects of the two stereoisomers of MDMA 3 h after drug administration. Previous studies have demonstrated that the S-(+) stereoisomer of MDMA is more potent than the R-(−) isomer, both in terms of dopamine release and neurotoxicity (Schmidt *et al.*, 1987). Although both enantiomers reduced striatal concentrations of 5-HT, 5-hydroxyindoleacetic acid and DOPAC, only S-(+) MDMA produced the acute elevation of dopamine concentrations observed with the racemic drug.

The next series of experiments were designed to directly determine whether interference with dopamine synthesis was responsible for the protective effects of the 5-HT_2 antagonists. If this were the case, 5-HT_2 antagonists should not prevent MDMA-induced neurotoxicity in the presence of an adequate supply of releasable dopamine. To create this condition, brain dopamine concentrations were augmented by administering L-dopa (100 mg/kg i.p.) with the peripheral decarboxylase inhibitor carbidopa (25 mg/kg i.p., 30 min before). MDMA (20 mg/kg s.c.) with or without (±)MDL 11,939 (5 mg/kg s.c.) was administered simultaneously with the L-dopa and the rats were sacrificed one week later. Results from this experiment are shown in figure 3. MDMA alone reduced cortical 5-HT concentrations to 65% of control. MDL 11,939 completely prevented the MDMA-induced deficit in transmitter concentrations ($P < .05$ *vs.* MDMA). However, in animals administered L-dopa, the protective effect of the 5-HT_2 antagonist was blunted such that even in the presence of the antagonist, MDMA reduced cortical 5-HT concentrations to 55% of control ($P < .05$ *vs.* saline or MDMA + MDL 11,939). In addition, when MDMA alone was administered to animals also receiving L-dopa, the subsequent deficit in 5-HT concentrations was markedly enhanced ($P < .001$ *vs.* MDMA alone).

To extend these findings, this experiment was repeated at a higher dose of MDMA (30 mg/kg) using the 5-HT_2 antagonist, ritanserin (5 mg/kg s.c.). The resultant changes in cortical 5-HT concentrations for this experiment are shown in figure 4. At this higher dose, MDMA reduced cortical 5-HT concentra-

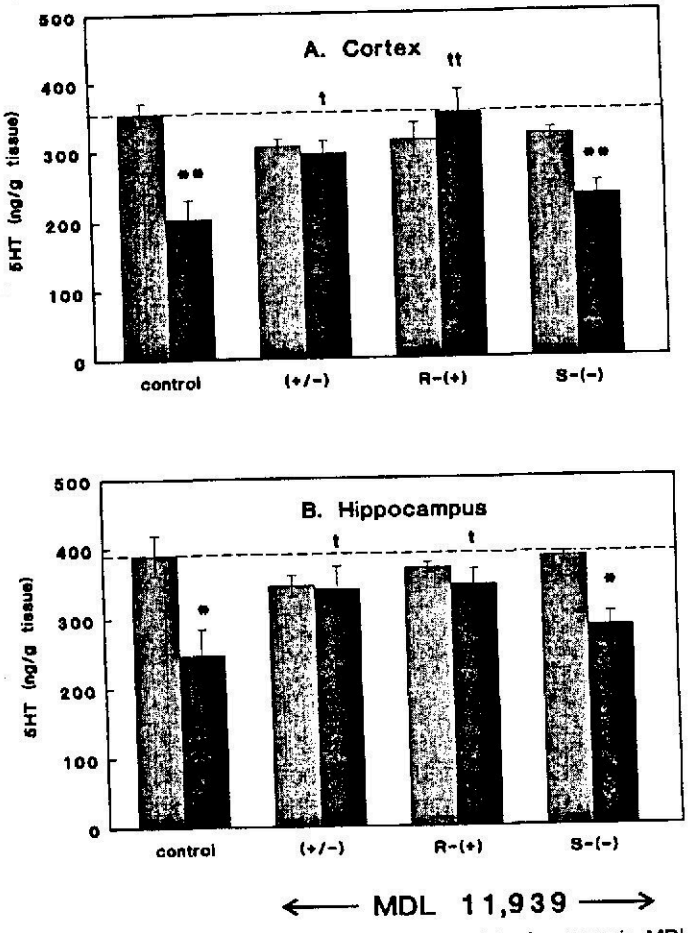

Fig. 2. Prevention of MDMA-induced neurotoxicity by racemic MDL 11,939 and its R-(+) enantiomer as determined by changes in cortical (A) and hippocampal (B) 5-HT concentrations. Drugs were administered simultaneously one week before sacrifice of the animals. Drug doses and indications of significance are described in figure 1.

TABLE 1

Effect of haloperidol or nomifensine on the acute MDMA-induced alteration in striatal dopamine metabolism

All drugs were administered simultaneously 3 h before sacrifice. Data are presented as the mean ± S.E.M. for five animals. All comparisons are made against the appropriate group in the absence of MDMA. **, *** = $P < .01, .001$ by the two-tailed Student's *t* test.

	Dopamine	DOPAC	HVA
		μg/g	
Saline	9.6 ± 0.2 (100%)	1.17 ± 0.06 (100%)	0.59 ± 0.03 (100%)
MDMA (10 mg/kg s.c.)	12.1 ± 0.3 (126%)***	0.80 ± 0.06 (69%)**	0.66 ± 0.05 (112%)
Haloperidol (2 mg/kg i.p.)	8.5 ± 0.4 (100%)	4.13 ± 0.08 (100%)	3.32 ± 0.18 (100%)
plus MDMA	11.0 ± 0.6 (129%)**	3.15 ± 0.222 (76%)**	3.49 ± 0.15 (105%)
Nomifensine (10 mg/kg s.c.)	10.5 ± 0.3 (100%)	1.16 ± 0.08 (100%)	1.09 ± 0.08 (100%)
plus MDMA	9.4 ± 0.5 (90%)	0.74 ± 0.05 (64%)**	0.92 ± 0.06 (84%)

TABLE 2

Acute effect of the stereoisomers of MDMA on striatal dopamine metabolism

Rats received either S-(+) or R-(−)MDMA (10 mg/kg s.c.) 3 h before sacrifice. Data are presented as the mean ± S.E.M. for five animals. *, **, *** = P < .05, .01, .001 *vs.* saline by the two-tailed Student's *t* test.

	Dopamine	DOPAC	HVA
		μg/g	
Saline	10.6 ± 0.6	1.64 ± 0.13	0.68 ± 0.08
	(100 ± 6.1)	(100 ± 8.1)	(100 ± 11.1)
S-(+)MDMA	13.1 ± 0.6*	0.830.05***	0.72 ± 0.07
	(124.3 ± 5.4)	(50.5 ± 3.3)	(105.2 ± 10.5)
R-(−)MDMA	9.4 ± 0.6	1.08 ± 0.05**	0.66 ± 0.04
	(89.2 ± 5.6)	(65.8 ± 3.4)	(96.5 ± 5.5)

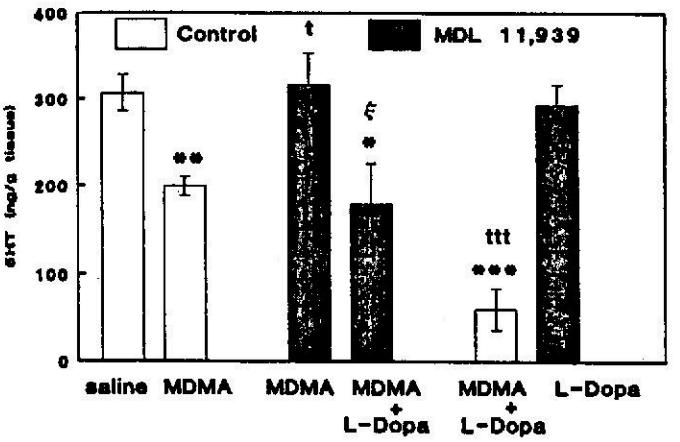

Fig. 3. L-dopa reversal of the prevention of MDMA-induced neurotoxicity by MDL 11,939. Rats treated with L-dopa (100 mg/kg i.p.) were given carbidopa (25 mg/kg i.p.) 30 min before. All other drugs were administered simultaneously. MDMA was given at 20 mg/kg s.c. and racemic MDL 11,939 was administered at 5 mg/kg s.c. Rats were sacrificed 1 week after drug administration. The results presented are for cortical 5-HT concentrations. *, **, *** = P < .05, .01, .001 *vs.* saline; t, ttt = P < .05, .001 *vs.* MDMA; ξ = P < .05 *vs.* MDMA + MDL 11,939.

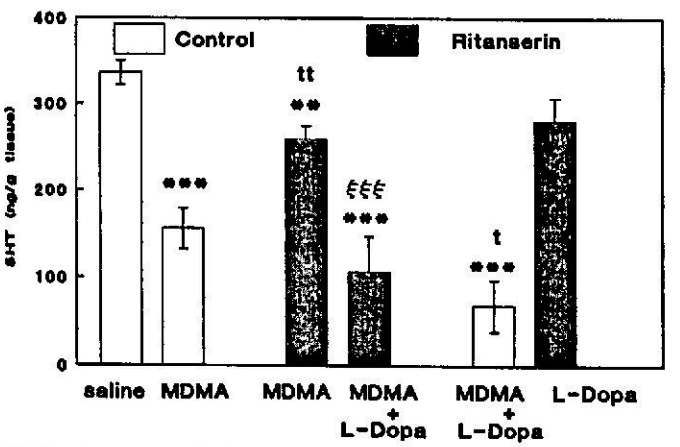

Fig. 4. L-dopa reversal of the prevention of MDMA-induced neurotoxicity by ritanserin. Ritanserin was administered at 5 mg/kg s.c. All other details are as described in figure 3. **, *** = P < .01, .001 *vs.* saline; t, tt = P < .05, .01 *vs.* MDMA; ξξξ P < .001 *vs.* MDMA + ritanserin.

tions to 46% of control (P < .001). This depletion again was amplified in L-dopa treated animals with cortical 5-HT concentrations being reduced to 20% of saline values (P < .05 *vs.* MDMA alone). Although the effect of MDMA in control animals was partially antagonized by ritanserin (P < .01), this protection was reversed by the administration of L-dopa. In this experiment the 5-HT$_2$ antagonist did not produce any significant reversal of the depletion in MDMA plus L-dopa animals.

Discussion

This study focused on the relationship between the acute effects of MDMA on the dopaminergic system and the long-term or neurotoxic effects of the drug. The first suggestion that these two phenomena might be linked came from the observation that both are sensitive to a number of 5-HT$_2$ antagonists (Nash *et al.*, 1990; Schmidt *et al.*, 1990b). In this study we have shown that only the stereoisomer of MDL 11,939 active at 5-HT$_2$ receptors altered the acute MDMA-induced changes in dopamine metabolism and prevented the long-term serotonergic deficits. Although some slight effect with the inactive S-(−) isomer of MDL 11,939 was observed, this may be attributable to an approximately 2% contamination with the R-(+) isomer.

These results demonstrate clearly that 5-HT$_2$ receptor activation plays a critical role in the neurochemical effects of MDMA. To understand how the acute and long-term interactions of the 5-HT$_2$ antagonists with MDMA are linked, several experiments were performed to determine the basis of the acute effect of MDMA on dopamine metabolism.

Amphetamines are known to have a variety of effects on the dopamine neuron. One of amphetamine's best described electrophysiological effects is the inhibition of cell firing in the nigrostriatal dopaminergic pathway (Bunney *et al.*, 1973). MDMA has recently been demonstrated to have a similar effect on dopaminergic cell firing which, like amphetamine, is sensitive to dopamine depletion with α-methyl-*p*-tyrosine or D2 receptor blockade with haloperidol (Kelland *et al.*, 1989). D2 receptor antagonists reverse the inhibition of cell firing due to amphetamine by blocking the activation of somatodendritic autoreceptors by dendritically released dopamine in the substantia nigra (Bunney *et al.*, 1973). More surprisingly, 5-HT$_2$ receptor antagonists have also been reported to reverse amphetamine-induced inhibition of cell firing (Goldstein *et al.*, 1989). In our own studies, this made it necessary to determine if inhibition of cell firing contributed to the rise in dopamine concentrations after MDMA because this would readily explain the reversal by MDL 11,939. However, since haloperidol did not block the MDMA-induced increase in striatal dopamine, this effect must be independent of the firing rate of the dopaminergic pathway. This leaves an increase in dopamine synthesis, which is known to occur after MDMA administration (Nash *et al.*, 1990), as the most probable cause of the rise in dopamine concentrations. Amphetamine has been shown to stimulate the synthesis of dopamine both *in vitro* (Connor and Kuczenski, 1986) and *in vivo* (Miller and Shore, 1982; Fung and Uretsky, 1982) through a mechanism sensitive to inhibitors of the dopamine uptake carrier. It is proposed that the carrier-mediated efflux of dopamine initiated by amphetamine depletes the newly synthesized or cytoplasmic pool of transmitter. This reduces feedback inhibition of tyrosine hydroxylase and causes a transient burst in dopamine synthesis (Butcher *et al.*, 1988). Like amphetamine, MDMA also releases dopamine *via* a carrier-dependent mechanism (Schmidt *et al.*, 1987) that has been shown to be sensitive to uptake blockers such as nomifensine *in vitro*. Thus, the ability of nomifensine to antagonize the acute elevation of striatal dopamine by MDMA *in vivo* is

consistent with this elevation being indicative of an increase in synthesis due to the release of dopamine by MDMA.

This particular experiment is relevant to another issue concerning MDMA's neurochemical effects. The mechanism through which uptake blockers interfere with the carrier-mediated release elicited by MDMA is controversial. A frequent explanation is that such inhibitors prevent the uptake of MDMA (Nash *et al.*, 1988). However, our observation that nomifensine does not alter the MDMA-induced decline in DOPAC concentrations indicates that the inhibitor does not prevent MDMA from gaining access to the interior of the nerve terminal. Similar results were reported by Fuller *et al.* (1979) in experiments using amphetamine and mazindol. Based on these results it seems likely that as a very lipophilic drug MDMA gains access to the interior of the neuron by passive diffusion where inhibition of monoamine oxidase may be responsible for the decline in DOPAC. Two earlier studies found no evidence that MDMA is actively transported into nerve terminals (Schmidt *et al.*, 1987; Wang *et al.*, 1987). It therefore seems most probable that nomifensine blocks carrier-mediated release by obstructing transmitter efflux.

The experiments with the stereoisomers of MDMA provide further evidence that the acute elevation of dopamine by MDMA is a response to carrier-mediated dopamine release. Both enantiomers of MDMA were previously shown to produce the acute decline in 5-HT (Schmidt *et al.*, 1987) and tryptophan hydroxylase activity (Schmidt and Taylor, 1988) described for the racemate. They are also similar in their potency at producing 5-HT release *in vitro* (Johnson *et al.*, 1986; Schmidt *et al.*, 1987). By contrast, the two stereoisomers differ in terms of their potency at producing both neurotoxicity and dopamine release. Only S-(+)MDMA produced any serotonergic deficits one week after administration of a single 10- or 20-mg/kg dose of either stereoisomer (Schmidt, 1987). Similarly, the (+) stereoisomer has been shown to be more potent at increasing the efflux of dopamine *in vitro*. Using microdialysis, Hiramatsu and Cho (1990) recently showed that R-(−)MDMA had virtually no effect on dopamine efflux *in vivo*. Our results show that only the (+) stereoisomer produced the acute rise in dopamine concentrations although both stereoisomers reduced DOPAC concentrations. This observation that only the neurotoxic enantiomer elevated dopamine concentrations lends support to the suggestion that this activation of dopamine synthesis is required for the long-term consequences of MDMA administration.

Based upon previous work indicating dopamine release is a necessity for MDMA-induced neurotoxicity (Stone *et al.*, 1988; Schmidt *et al.*, 1990a), these results provide an explanation for the protection provided by 5-HT_2 antagonists. The changes in dopamine metabolites observed after the administration of MDMA plus a 5-HT_2 antagonist indicate that transmitter release may be compromised in the absence of an increase in dopamine synthesis. The enhanced MDMA-induced decline in DOPAC in the presence of MDL 11,939 suggests that cytoplasmic dopamine levels are severely depleted under these conditions. Because cytoplasmic dopamine provides the substrate for carrier-mediated efflux as well as oxidation by monoamine oxidase (Parker and Cubeddu, 1986; Zetterstrom *et al.*, 1986), both processes will be affected. This is compatible with the observation that in the presence of 5-HT_2 receptor blockade, MDMA administration causes a large decline in the extraneuronal metabolism of dopamine as seen by the decrease in HVA concentrations.

If the protective effect of the 5-HT_2 antagonists was due to interference with the maintenance of the pool of dopamine released by MDMA, then providing an alternative source of dopamine should circumvent the effect of the antagonists. As demonstrated with both MDL 11,939 and ritanserin, satisfying the requirement for newly synthesized dopamine through the administration of L-dopa nullified the protective effects of the 5-HT_2 antagonists. Thus, the ability 5-HT_2 antagonists to block MDMA-induced deficits in the serotonergic nervous system is due to indirect interference with MDMA-mediated dopamine release.

The observation that coadministration of L-dopa with MDMA potentiated the subsequent serotonergic deficits also supports the hypothesis that dopamine release plays an obligatory role in MDMA-induced neurotoxicity. The extent of the serotonergic deficit in L-dopa-treated animals was at least twice that observed in animals given MDMA alone. It is worth mentioning that the behavioral effects of MDMA (locomotion, headweaving and sniffing) were also greatly augmented in the L-dopa-treated rats.

In summary, accepting the premise that dopamine release is causal in MDMA neurotoxicity, the results of this study provide an explanation of the mechanism through which antagonists at 5-HT_2 receptors can prevent these long-term effects of MDMA. Independent of these findings, the observation that L-dopa potentiated the serotonergic deficits produced by MDMA is additional support for the hypothesis that MDMA-induced neurotoxicity is a consequence of MDMA-induced dopamine release. Finally, these results also demonstrate a pharmacologically important interaction between the serotonergic and dopaminergic systems. Although the serotonergic input to the substantia nigra is generally considered to have an inhibitory effect on the dopamine system, there are conditions under which it appears that 5-HT release may be necessary for the maintenance of dopaminergic activity and that 5-HT_2 receptors mediate this process.

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