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Antagonism of the neurotoxicity due to a single administration of methylenedioxymethamphetamine

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The role of transmitter release in the serotonergic neurotoxicity of methylenedioxymethamphetamine (MDMA) was examined using treatments altering MDMA-induced release or its consequences. The long-term decrease in 5-HT concentrations and tryptophan hydroxylase activity produced by MDMA was antagonized by depletion of vesicular monoamines with reserpine or interruption of monoamine synthesis with the decarboxylase inhibitor, monofluoromethyl DOPA (dihydroxyphenylalanine). Similar results were achieved by selectively inhibiting dopamine synthesis with α-methyl-p-tyrosine or through bilateral lesions of the substantia nigra with 6-hydroxydopamine. The dopamine receptor antagonist haloperidol was also effective in this regard. Although these results strongly implicate dopamine release in the long-term neurochemical effects of MDMA, protection was also provided by selective 5-HT_2 antagonists indicating that the neurotoxicity is dependent upon the release of both dopamine and 5-HT.

MDMA (methylenedioxymethamphetamine); 5-HT (5-hydroxytryptamine, serotonin); Dopamine neurotoxicity

1. Introduction

Methylenedioxymethamphetamine (MDMA) is a psychedelic amphetamine analogue known to produce a selective degeneration of serotonergic nerve terminals in laboratory animals (Schmidt et al., 1986; Stone et al., 1986; Schmidt, 1987). This property and concern over its widespread abuse have aroused a great deal of interest in the drug as it relates to the neurotoxicity of amphetamines in general. Numerous studies have now demonstrated that the administration of MDMA to rats results in a persistent reduction in central 5-HT concentrations and a loss in the activity of the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH) (Stone et al., 1986; Schmidt and Taylor, 1987; 1988). This decline in neurotransmitter concentrations correlates with a reduction in the number of 5-HT uptake sites as shown both biochemically (Schmidt, 1987) and through ligand binding studies (Battaglia et al., 1987). Although such changes in serotonergic markers can be observed after a single high dose of MDMA, indices of dopaminergic function show only transient alterations even after multiple drug administrations (Stone et al., 1987).

Both in vitro (Johnson et al., 1986; Schmidt et al., 1987) and in vivo (Yamamoto and Spanos, 1988) studies have shown MDMA to have potent effects on monoamine release. Similar to other amphetamine analogs, this release occurs through a Ca^{2+}-independent, carrier-mediated mechanism. Consistent with its selective long-term effects, MDMA is more potent at inducing the release of 5-HT rather than catecholamines (Johnson et al., 1986; Schmidt et al., 1987). This is one of many characteristics MDMA shares with another

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serotonergic neurotoxin p-chloroamphetamine (PCA).

We have previously demonstrated that inhibitors of the 5-HT uptake system antagonize MDMA-induced 5-HT release in vitro (Schmidt et al., 1987) and the neurotoxicity of MDMA in vivo (Schmidt, 1987). Similar studies have implicated carrier-mediated processes in the neurotoxic effects of methamphetamine (METH) (Schmidt and Gibb, 1985a,b) and amphetamine (Fuller and Hemrick-Luecke, 1980; Steranka, 1982). What has remained unresolved is whether interference with the uptake carrier is affecting neurotoxicity by blocking carrier-mediated release of transmitter or the uptake of some molecule which initiates the neurotoxicity. Several studies have implicated the carrier-mediated uptake of dopamine or an oxidation product of dopamine in the destruction of serotonergic and dopaminergic nerve terminals following the administration of METH (Seiden and Vosmer, 1984; Schmidt et al., 1985; Johnson et al., 1987).

The demonstrated carrier-dependent neurotoxicity of MDMA suggests monoamine release may be involved in this phenomenon as well. We have therefore examined how treatments modifying monoaminergic function affect the neurotoxicity measured 1 week following a single s.c. administration of MDMA (20 mg/kg). This regimen has previously been demonstrated to produce a significant decrement in the number of high affinity 5-HT uptake sites in the rat brain (Schmidt, 1987). Furthermore, the acute neurochemical effects of MDMA have waned by 1 week allowing a determination of the effect of any treatment selectively on the neurotoxicity (Schmidt, 1987).

2. Materials and methods

2.1. Drug administration

For each experiment, male Sprague-Dawley rats (300-500 g) were divided into groups of five and maintained on a 12 h light-dark cycle with free access to food and water. MDMA was administered s.c. as the free base in saline. All other drugs were administered as the salt. Animals were killed by decapitation and the brains were rapidly dissected on ice. Tissues were stored at $-70\,^{\circ}C$ until assayed.

MDMA was provided by the National Institute on Drug Abuse. Reserpine, α-methyl-p-tyrosine (AMPT), prazosin and PCA were purchased from Sigma Chemical Co. (St. Louis, MO). Ketanserin and haloperidol were from Research Biochemicals Inc. (Natick, MA). Pindolol was the generous gift of Sandoz Pharmaceuticals (Basel, Switzerland). MDL 11,939, MDL 26,508, MDL 73,147 and monofluoromethyl DOPA (dihydroxyphenylalanine) were synthesized at the Merrell Dow Research Institute.

2.2. Determination of tryptophan hydroxylase activity and 5-HT concentrations

TPH activity was assayed by the coupled $^{14}CO_2$ trapping method described previously (Hotchkiss et al., 1979; Schmidt and Taylor, 1987) using aromatic L-amino acid decarboxylase partially purified from bovine kidney (Lovenberg and Engelman, 1971). Brain concentrations of 5-HT, dopamine and their metabolites were determined by high performance liquid chromatography with electrochemical detection as described previously (Schmidt and Taylor, 1988).

2.3. Substantia nigra lesions with 6-hydroxydopamine (6-DHDA)

Rats maintained under methoxyflurane anesthesia were positioned in a Kopf model 900 small animal stereotaxic apparatus. Small burr holes were made through the skull above the injection site and a fine needle was lowered to the coordinates -5.3, ± 2.0, -7.0 mm relative to bregma (Paxinos and Watson, 1982). 6-OHDA (12 μg) was infused over a 5 min period in 5 μl of 1% ascorbic acid. The needle was left in place for an additional 5 min before its removal and closure of the burr hole with bone wax. Sham-lesioned rats had the needle lowered to the proper coordinates and immediately removed. For bilateral lesions, the animals were allowed to recover for 5-7 days prior to lesioning of the contralateral nigra. All animals were allowed for recover for 1 week after

lesioning before the administration of MDMA or PCA. Sacrifice was 1 week following MDMA or PCA administration.

3. Results

3.1. Synthesis and storage inhibitors

Vesicular storage of both catecholamines and indolamines was disrupted by administering reserpine (5 mg/kg s.c.) to rats 18 h prior to MDMA. TPH activity was used as the marker for MDMA-induced neurotoxicity in these experiments since 5-HT concentrations were still depressed 1 week after this pretreatment (fig. 1A). Although MDMA did not reduce enzyme activity to the same extent in reserpine-pretreated rats as in those treated with MDMA alone (fig. 1B), an elevation in TPH activity by reserpine made it difficult to evaluate this apparent antagonism. To avoid this confounding effect of reserpine, the experiment was repeated and the animals were allowed to survive for 2 weeks. The results for cortical and hippocampal TPH activity are provided in fig. 1C, D, respectively. Under these conditions reserpine did not affect TPH activity but did antagonize the loss of enzyme activity produced in both regions by MDMA. An effect on 5-HT concentrations was again obscured by a significant reserpine-induced depletion (data not shown).

Amphetamines are believed primarily to release monoamines from a newly synthesized pool of transmitter which may be distinct from that depleted by reserpine. To affect the newly synthesized pool specifically, we selected the irreversible inhibitor of L-aromatic amino acid decarboxylase, monofluoromethyl DOPA (MFMD). The drug was administered for 3 consecutive days at a dose of 100 mg/kg i.p. On the third day the animals were given either saline or MDMA (20 mg/kg). Half of the animals were killed 3 h following MDMA

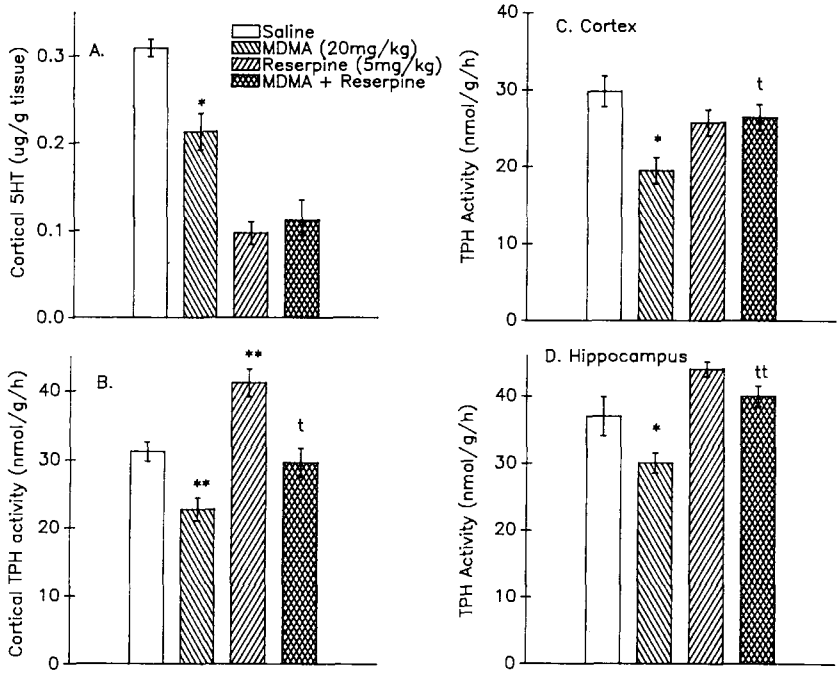

Fig. 1. Effect of reserpine pretreatment on MDMA-induced neurotoxicity. Rats were administered reserpine 18 h prior to MDMA and sacrificed 1 (A and B) or 2 weeks (C and D) later. Both cortical 5-HT concentrations (A) and TPH activity (B) are displayed for the earlier time point while the results for cortical (C) and hippocampal (D) TPH activity are displayed for the 2 week time point. Data are presented as the means ± S.E.M. for five or more animals. * $P < 0.05$, ** $P < 0.01$ compared to control; $^{t}P < 0.05$, $^{tt}P < 0.01$ compared to MDMA alone.

TABLE 1

The effect of MFMD and MDMA on striatal monoamine and metabolite concentrations at 3 h. Rats were pretreated for 3 days with MFMD (100 mg/kg i.p.). On day 4 either saline or MDMA (10 mg/kg s.c.) was administered 3 h prior to sacrifice. [a] $P < 0.05$ vs. saline by ANOVA/SNK. N.D. = not detected.

	Saline ± S.E.M. μg/g	MFMD ± S.E.M. μg/g	MDMA ± S.E.M. μg/g	MFMD + MDMA + S.E.M. μg/g
Dopamine	10.220 ± 0.260	4.740 ± 1.490 [a]	11.970 ± 0.550	3.710 ± 1.840 [a]
	(100 ± 2.5)	(46.4 ± 14.6)	(117.1 ± 5.4)	(36.3 ± 18.0)
DOPAC	1.060 ± 0.039	0.859 ± 0.073 [a]	0.795 ± 0.066 [a]	0.607 ± 0.050 [a]
	(100 ± 3.7)	(81.0 ± 6.9)	(75.0 ± 6.2)	(57.3 ± 4.7)
HVA	0.732 ± 0.050	0.174 ± 0.108 [a]	0.733 ± 0.056	0.052 ± 0.052 [a]
	(100 ± 6.8)	(23.8 ± 14.8)	(100.1 ± 7.7)	(7.1 ± 7.1)
5-HT	0.454 ± 0.011	0.144 ± 0.088 [a]	0.169 ± 0.028 [a]	N.D. [a]
	(100 ± 2.4)	(31.7 ± 19.4)	(37.2 ± 6.2)	
5-HIAA	0.440 ± 0.011	0.058 ± 0.037 [a]	0.315 ± 0.032	0.114 ± 0.080 [a]
	(100 ± 2.5)	(13.2 ± 8.4)	(71.6 ± 7.3)	(25.9 ± 18.2)

administration to allow a determination of the effect of the treatment on the acute neurochemical changes produced by MDMA. The remaining animals were killed 1 week later to determine the effect on MDMA-induced neurotoxicity.

Table 1 provides the results from the de-

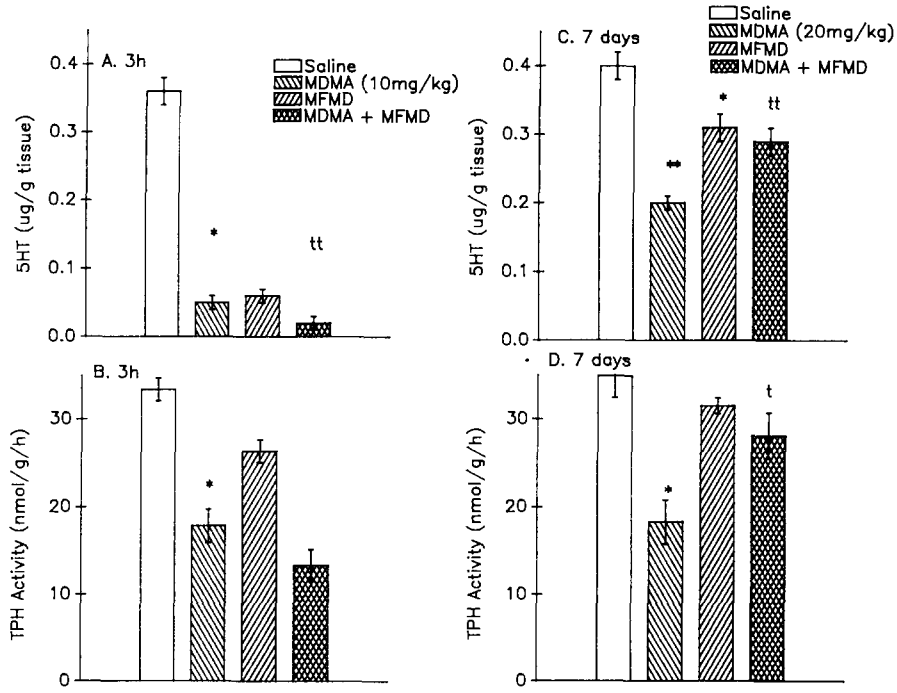

Fig. 2. Effect of aromatic L-amino acid decarboxylase inhibition by MFMD on both the acute (A and B) and long-term (C and D) neurochemical effects of MDMA. MFMD (100 mg/kg s.c.) was given daily for 3 days prior to MDMA. Cortical 5-HT and TPH activity are shown for both the 3 h and 1 week time point. Data are presented as the means ± S.E.M. for five or more animals * $P < 0.05$, ** $P < 0.01$ compared to MDMA alone.

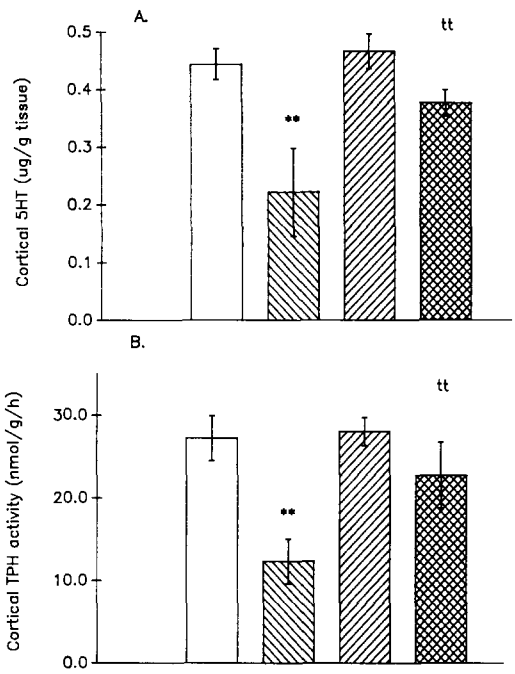

Fig. 3. Antagonism of the long-term neurochemical effects of MDMA on cortical 5-HT and TPH activity by AMPT pretreatment. AMPT was given 3 h prior to and with MDMA. Decapitation was at 1 week. Data are presented as the means ± S.E.M. for five or more animals. ** $P < 0.01$ compared to control; $^{tt}P < 0.01$ compared to MDMA alone. □ Saline; ▧ MDMA (20 mg/kg); ▨ AMPT (2 × 100 mg/kg); ■ MDMA + AMPT.

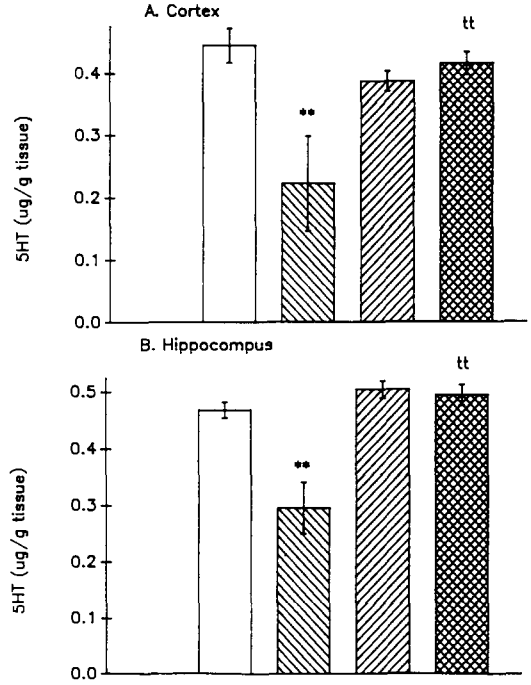

Fig. 4. Blockade of the MDMA-induced decrease in cortical and hippocampal 5-HT concentrations by coadministration of haloperidol. Animals were killed 1 week following drug administration. Data are presented as the means ± S.E.M. for five or more animals. ** $P < 0.01$ compared to control; $^{tt}P < 0.01$ compared to MDMA alone. □ Saline; ▧ MDMA (20 mg/kg); ▨ haloperidol (2 mg/kg); ■ MDMA + haloperidol.

termination of striatal monoamine and metabolite concentrations at the 3 h time point. MFMD reduced striatal dopamine and 5-HT concentrations to 46 and 32% of control, respectively. The concentration of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) declined only 19% indicating that dopamine turnover itself was not affected greatly by depletion of the parent amine. In contrast, homovanillic acid (HVA) concentrations decreased to 24% of control suggesting most of the dopamine released is taken back up by dopaminergic terminals under these conditions. MDMA produced a small elevation of dopamine and a reduction in DOPAC concentrations as previously reported (Schmidt et al., 1986). 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were also reduced significantly. The combination of the two drugs produced additive effects on 5-HT to the extent that striatal 5-HT concentrations were below detectable levels in these animals. Figure 2A shows a similar additivity in the effect of the two drugs on cortical 5-HT concentrations. The depletion of monoamines by MFMD was also without effect on the acute loss of cortical TPH activity produced by MDMA (fig. 2B).

In contrast to the lack of effect of MFMD pretreatment on the acute changes produced by MDMA, the results at 1 week indicate that decarboxylase inhibition provided protection against the neurotoxicity. Both the depletion of cortical 5-HT and the loss of TPH activity (fig. 2C,D) were significantly attenuated by MFMD pretreatment. MDMA reduced cortical 5-HT concentrations and TPH activity to 49 and 52% of control, respectively, while these same values were 72 and

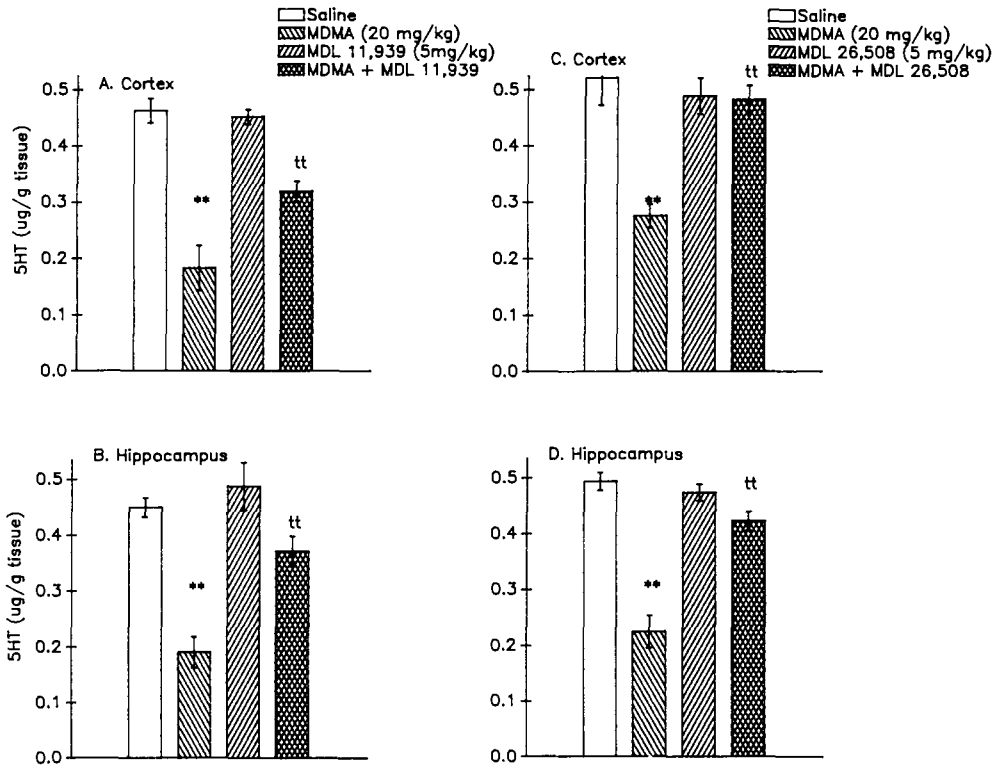

Fig. 5. Effect of 5-HT_2 receptor antagonists on the long-term depletion of 5-HT produced by MDMA. MDL 11,939 and MDL 26,508 were coadministered with MDMA 1 week prior to decapitation. Data are presented as the means ± S.E.M. for five or more animals. ** $P < 0.01$ compared to control; $^{tt}P < 0.01$ compared to MDMA alone.

80% in pretreated animals. Similar results were observed in the hippocampus and in a second experiment.

The competitive inhibitor of tyrosine hydroxylase, AMPT was used to determine if selective depletion of the newly synthesized pool of dopamine would alter the long-term neurochemical consequences of MDMA. The inhibitor was given at 100 mg/kg 3 h prior to and with MDMA. The results of this experiment are displayed in fig. 3. MDMA alone reduced cortical 5-HT concentrations to 50% of control; however, in combination with AMPT, MDMA reduced cortical 5-HT values to only 85% of control ($P < 0.01$). One week after MDMA, cortical TPH activity was reduced to 45% of control while in AMPT pretreated animals this value was 84% ($P < 0.01$). Similar results were observed in the hippocampus (data not shown).

3.2. Receptor antagonists

Figure 4 shows the cortical and hippocampal 5-HT concentrations from an experiment in which the dopamine receptor antagonist, haloperidol (2 mg/kg, i.p.) was coadministered with MDMA 1 week prior to sacrifice. Haloperidol completely blocked depletions of the transmitter in both regions ($P < 0.01$). The loss of cortical and hippocampal TPH activity was also completely antagonized by haloperidol (data not shown). Similar results were obtained in a duplicate experiment.

Figure 5 provides results from experiments in which the selective 5-HT_2 antagonists MDL 11,939 (Dudley et al., 1988) or MDL 26,508 (Dudley et al., in preparation) were coadministered with MDMA. Both compounds produced a highly significant ($P < 0.01$) antagonism of the neurotoxic

effect of MDMA as measured by 5-HT concentrations in the cortex (fig. 5A,C) and hippocampus (fig. 5B,D). The corresponding loss of cortical and hippocampal TPH activity was also completely blocked by the two antagonists (data not shown). In vitro experiments with MDL 26,508 did not detect any effect on 5-HT uptake indicating blockade of neurotoxicity was due to blockade of the 5-HT_2 receptor (data not shown).

In related experiments, several other receptor antagonists were found to be ineffective in altering the long-term neurochemical response to MDMA in either the cortex or hippocampus. The data for cortical 5-HT concentrations are listed in table 2. Neither the α_1-receptor antagonist prazosin (3 mg/kg) nor pindolol (5 mg/kg), a β-adrenoceptor antagonist and nonselective antagonist of 5-HT_1 receptors (Tricklebank, 1985), had any effect on MDMA-induced neurotoxicity. A similar lack of effect was observed with the 5-HT_3 antagonist, MDL 73,147 (5 mg/kg) (Gittos and Fatoni, in press).

TABLE 2

Lack of effect of various receptor antagonists on the long-term depletion of cortical 5-HT concentrations by MDMA. [a] $P < 0.01$ compared to saline.

Antagonist	Treatment			
	Saline	MDMA	Antagonist	MDMA + antagonist
Prazosin	0.52 ± 0.05 (100)	0.28 ± 0.02 [a] (54)	0.53 ± 0.01 (102)	0.28 ± 0.03 (53)
Pindolol	0.44 ± 0.01 (100)	0.32 ± 0.03 [a] (72)	0.43 ± 0.01 (99)	0.36 ± 0.06 (81)
MDL 73,147	0.44 ± 0.01 (100)	0.32 ± 0.03 [a] (72)	0.45 ± 0.03 (101)	0.32 ± 0.02 (73)

3.3. *Lesions studies*

Figure 6 shows the effect of unilateral lesions of the substantia nigra on the neurotoxicity of MDMA or the closely related amphetamine analogue, PCA. The drugs were administered 1 week

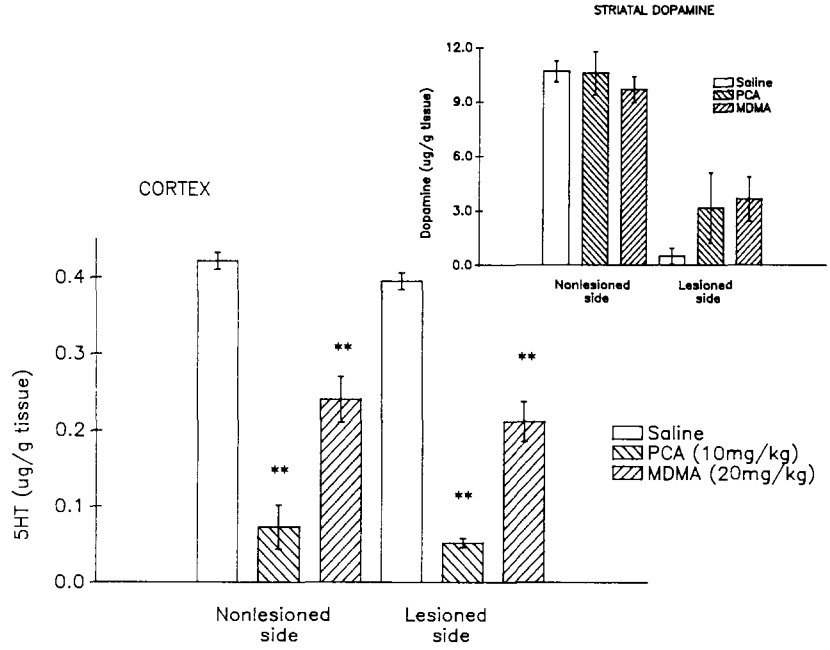

Fig. 6. Lack of effect of unilateral lesions of the substantia nigra with 6-OHDA on the neurotoxic effect of PCA or MDMA. Data are presented as the means ± S.E.M. for five or more animals. Animals were lesioned 1 week prior to drug administration and 2 weeks prior to sacrifice. The effect of the lesions on striatal dopamine concentrations is shown in the inset. ** $P < 0.01$ compared to control.

after the 6-OHDA treatment and 1 week prior to decapitation. As shown in the insert, 6-OHDA depleted striatal dopamine on the lesioned side to less than 10% of the control side in the saline-treated rats. In spite of this depletion, cortical 5-HT concentrations on both sides were reduced to the same extent by either MDMA or PCA. This experiment was repeated using bilaterally lesioned animals with average dopamine depletions on each side of the brain of approximately 70% (fig. 7A). In contrast to the effect of the unilateral lesions, destruction of the dopamine cell bodies in both substantia nigra significantly attenuated the MDMA-induced depletion of cortical (fig. 7B) and hippocampal (fig. 7C) 5-HT concentrations ($P < 0.01$). Similar results were observed in a second experiment.

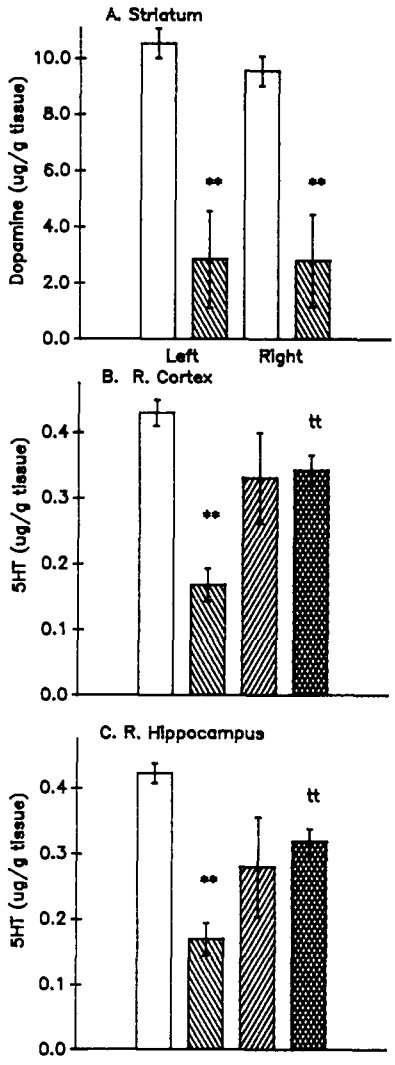

Fig. 7. Antagonism of the neurotoxic effect of MDMA by bilateral 6-OHDA lesions of the substantia nigra. Animals were given 1 week to recover between each surgery. MDMA was administered 1 week following the last surgery and 1 week prior to sacrifice. Data are presented as the means ± S.E.M. for five or more animals. ** $P < 0.01$ compared to control; $^{tt}P < 0.01$ compared to MDMA alone. (A) □ Control; ▨ 6-OHDA. (B, C) □ Saline; ▨ MDMA (20 mg/kg); ▨ 6-OHDA; ▩ MDMA + 6-OHDA.

4. Discussion

Despite a significant research effort, the mechanism(s) responsible for the long-term neurochemical effects of the amphetamines has eluded discovery for several decades. Although the recent surge in the recreational use of MDMA has made it the newest member of this class to receive such scrutiny, it is by no means unique. PCA, which has a neurochemical profile virtually indistinguishable from MDMA, is often used as a tool for selectively ablating serotonergic terminals. Similarly, the neurochemical sequelae of high doses of METH or amphetamine in rats have been known for some time (Fibiger and McGeer, 1971; Koda and Gibb, 1973; Ellison et al., 1978) while the persistent depletion of rat brain 5-HT by fenfluramine was reported over a decade ago (Harvey and McMaster, 1975; Sanders-Bush et al., 1975).

One of the interesting features of the neurotoxicity produced by high dose of amphetamines is the pattern or specificity of the response. Thus, in contrast to the effects of PCA or MDMA, high doses of amphetamine produce persistent changes only in the dopaminergic system (Fuller and Hemrick-Luecke, 1982; Peat et al., 1985). Intermediate between these two extremes is methamphetamine which is neurotoxic to both the serotonergic and dopaminergic systems at high doses (Hotchkiss and Gibb, 1980; Wagner et al., 1980; Trulson and Trulson, 1982). One common feature of these drugs is their mechanism of behavior activation through an increase in the car-

rier-mediated efflux of transmitter. It is plausible that this common denominator is involved in the neurotoxicity produced by these drugs as well. This hypothesis is supported by the observation that drugs known to interfere with the carrier-mediated release elicited by amphetamines also block amphetamine-induced neurotoxicity. Unfortunately, the interpretation of these results has been clouded by the fact that these same drugs may be interfering with the uptake of the amphetamine itself (Fuller, 1980).

The potential link between the monoamine release produced by MDMA and its neurotoxicity was tested in several ways. Our initial experiments focused on treatments which would affect all monoaminergic neurotransmission such as pretreatment with reserpine or MFMD. As previously reported by Stone et al. (1988), monoamine depletion by reserpine significantly antagonized the long-term loss of TPH activity produced by a single administration of MDMA. This observation provides evidence that some critical monoamine concentration is required for the neurotoxic response to MDMA; however, it does not indicate if a specific transmitter system is involved. The protection observed following MFMD pretreatment is similar in this regard in that decarboxylase inhibition affected the concentrations of both 5-HT and dopamine. An advantage in the use of MFMD is that the more transient depletions produced by the decarboxylase inhibition allow 5-HT concentrations to be used as a marker of neurotoxicity. Additionally, and unlike reserpine, the effects of decarboxylase inhibition would be manifested in the newly synthesized pool of transmitter which is most susceptible to release by amphetamine-like agents (Parker and Cubeddu, 1986). As previously demonstrated using reserpine, depletion of monoamines by MFMD did not alter the acute effect of MDMA on either 5-HT or TPH activity (Schmidt and Taylor, 1987).

The importance of the newly synthesized pool of dopamine has been clearly demonstrated for METH where the blockade of METH-induced neurotoxicity by AMPT can be completely reversed by administration of the dopamine precursor, L-DOPA (Gibb and Kogan, 1979; Schmidt et al., 1985). Stone et al. (1988) have recently reported that AMPT can also partially antagonize the neurochemical deficits produced by MDMA at 3 days. The dopamine uptake inhibitor GBR 12909 was also shown to be protective in this study, presumably by interfering with carrier-mediated dopamine release. Our own results with AMPT are consistent with this observation and strongly suggest a role for dopamine in these MDMA-induced changes. The protective effect of haloperidol and that provided by the bilateral 6-OHDA lesions are also consistent with this suggestion.

In spite of the weight of the evidence that dopamine release is somehow involved in or can modify the neurotoxic effects of MDMA, it is difficult to describe a mechanism of neurotoxicity which can explain all the findings reported here. If, as has been suggested for METH (Schmidt et al., 1985; Johnson et al., 1987), dopamine is accumulated by 5-HT terminals where it directly or indirectly produces oxidative damage, why does a receptor antagonist such as haloperidol prevent this? The protection provided by reserpine is also surprising since its effects would not be on the amphetamine releasable pool of dopamine. It is worth noting that in contrast to its effects on MDMA, reserpine has been reported to exacerbate the long-term neurochemical effects of METH (Wagner et al., 1982). Perhaps the most inexplicable observation is the requirement for bilateral lesions of the substantia nigra to block the neurotoxicity of MDMA. Finally, how could the blockade of 5-HT_2 receptors protect against MDMA neurotoxicity? We are unaware of any effect of 5-HT antagonists on the neurotoxicity due to METH. Although there are presently no definitive answers to these questions some speculation is possible.

While there are certainly some clear differences in the neurotoxicity of the various amphetamines discussed here, it seems unlikely each would have its own unique mechanism for causing the degeneration of monoaminergic nerve terminals. This is particularly true considering that some of the same treatments antagonize the neurotoxicity of both MDMA and METH. It seems plausible that the slight differences in these compounds that result in their individual patterns of neurotoxicity could result in differences in the susceptibility of each

compound to various interventions. This would mean that in addition to manipulations directly influencing the primary mechanism of neurotoxicity, other perturbations in the involved neurons could also alter an activity of MDMA sufficiently to antagonize its long-term neurochemical effects. Such an effect may be the basis of the protection provided by the 5-HT_2 antagonists in this report. Activation of 5-HT receptors is known to be a consequence of MDMA administration in rats (Schechter, 1986; 1989) and this activation may contribute to the neurochemical environment required to produce damage to the serotonergic terminal. For example, recent studies conducted in our laboratory indicate that 5-HT_2 receptor blockers can antagonize the hyperthermia produced by MDMA (Schmidt and Taylor, in preparation). While the role of hyperthermia in the neurotoxicity of amphetamines is not known, interference with this activity could alter the metabolism or disposition of the drug in such a way as to disrupt its long-term neurochemical effects. The hypothermia produced by reserpine could have a similar effect. Studies are now in progress to test the role of hyperthermia in the neurotoxicity of MDMA.

Based on the available data, it is necessary to describe the mechanism of MDMA-induced neurotoxicity as dependent on the activation of 5-HT_2 receptors during a period of carrier-mediated dopamine release. Since MDMA releases 5-HT through a carrier-mediated mechanism, this suggests that the carrier-mediated release of both dopamine and 5-HT is involved in the neurotoxicity of MDMA. The ultimate cause of the degeneration of the serotonergic terminal is still unknown. The potential role of dopamine as a mediator of oxidative damage following high dose administration of amphetamines has been previously elaborated on (Schmidt et al., 1985) and has been discussed more recently by Stone et al. (1988). Although the ability of the sulfhydryl agent, cysteine, to antagonize the long-term loss of 5-HT following PCA (Steranka and Rhind, 1987) or MDMA (Schmidt and Taylor, unreported observation) supports the suggestion that oxidative stress may be involved in the neurotoxicity of amphetamines; further work on this hypothesis is required.

In summary, we have demonstrated that manipulations selected to block MDMA-induced monoamine release or its consequences, can interfere with the neurotoxicity due to a single administration of MDMA. Significant antagonism of MDMA-induced neurotoxicity was observed following the reductions in monoamine concentrations produced by depletion of either vesicular stores with reserpine or of the newly synthesized pool by decarboxylase inhibition. Selective inhibition of dopamine synthesis by AMPT was also effective in this regard. Protection against the serotonergic deficits produced by MDMA was also provided after bilateral but not unilateral 6-OHDA lesions of the substantia nigra. Haloperidol and several 5-HT_2 receptor antagonists also blocked the neurotoxicity of MDMA. The results indicate a complex mechanism of neurotoxicity dependent upon the carrier-mediated release of both dopamine and 5-HT.

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