Serotonin Neurotoxicity in Rats After Combined Treatment With a Dopaminergic Agent Followed by a Nonneurotoxic 3,4-Methylenedioxymethamphetamine (MDMA) Analogue

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JOHNSON, M. P., X. HUANG AND D. E. NICHOLS. *Serotonin neurotoxicity in rats after combined treatment with a dopaminergic agent followed by a nonneurotoxic 3,4-methylenedioxymethamphetamine (MDMA) analogue.* PHARMACOL BIOCHEM BEHAV **40**(4) 915–922, 1991.—There is increasing evidence linking dopamine (DA) to the long-term serotonergic (5-HT) neurotoxic effects of certain substituted amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA). The present study was undertaken to examine the importance of DA metabolism, uptake inhibition and release in the long-term effects of these drugs by combining various dopaminergic agents with an analogue of MDMA that had low neurotoxic liability, namely 5,6-methylene-dioxy-2-aminoindan (MDAI). Monoamine and metabolite levels and the number of 5-HT uptake sites (using [^{3}H]paroxetine binding) were determined 3 hours or 1 week after treatments. Combining the monoamine oxidase inhibitors, clorgyline (MAO$_A$ selective) or deprenyl (MAO$_B$ selective) with MDAI did not result in any long-term reductions of serotonergic markers. Similarly, combining the DA uptake inhibitor GBR-12909 with MDAI did not result in any long-term changes in monoamine levels at 1 week. In contrast, a single pretreatment or posttreatment with the nonvesicular DA releaser S-amphetamine and MDAI resulted in small but significant long-term changes in monoamine levels. More importantly, if a subacute dosing regimen (every 12 hours for 4 days) was utilized, the combination of S-amphetamine with MDAI resulted in a marked long-term decrease in the levels of cortical, hippocampal and striatal 5-HT, 5-HIAA and the number of 5-HT uptake sites. The results are discussed in terms of the significance of DA and especially nonvesicular DA release in the long-term effects of MDMA-like drugs.

MDMA   Dopamine   Neurotoxicity   Serotonin   Amphetamine   Monoamine oxidase inhibitors

THE long-term effects of 3,4-methylenedioxymethamphetamine (MDMA) and its primary amine homologue, 3,4-methylene-dioxyamphetamine (MDA), have been intensively studied in the past five years [for a review, see (21)]. It is now fairly well accepted that single or multiple high doses of MDA or MDMA result in long-term decreases in serotonin (5-HT) (3, 4, 7, 27, 38, 43, 46, 49) and serotonergic markers such as the number of [^{3}H]paroxetine binding sites (3, 4, 18, 30). Subsequent work has indicated that these decreases are due to the selective degeneration of the fine axons originating from the dorsal raphe nucleus (28, 31, 50). These neurodegenerative effects of MDA and MDMA are similar to those seen with other substituted amphetamines such as *p*-chloroamphetamine (PCA) (9, 11, 22, 24) and fenfluramine (10, 19, 28, 45).

Much of the work with MDMA and MDMA-like drugs has

suggested that dopamine (DA) may play an important role in the serotonergic neurotoxicity. For example, a positive correlation between the relative ability of MDMA-like drugs to induce DA release in vitro and their ability to induce a neurotoxic response in vivo has been previously noted. This is true for PCA (17, 30, 41), the enantiomers of MDA and MDMA (13, 16, 37, 38, 41, 44), the N-ethyl (12, 37, 46) and α-ethyl (16, 18, 44) analogues of MDMA, as well as a number of rigid analogues of MDMA (14, 15, 30).

Furthermore, pretreatment with a number of dopaminergic agents will either block or attenuate the long-term neurotoxic effects of MDMA. For instance, DA uptake inhibitors such as GBR-12909 attenuate the MDMA-induced persistent changes in serotonergic markers (47). Depleting DA stores with α-methyl-*p*-tyrosine attenuates the long-term serotonergic effects of MDMA

in the striatum, hippocampus and cortex (40,47). Similarly, decreasing catecholamine stores with either reserpine (40,47) or the decarboxylase inhibitor monofluoromethyl-DOPA (40) attenuates or blocks the persistent serotonergic deficits induced by MDMA. Furthermore, selectively induced lesions of the nigrostriatal dopaminergic system with bilateral injections of 6-hydroxydopamine in the substantia nigra block the long-term effects of MDMA (40,47). Therefore, much of the evidence would seem to suggest that DA plays an important role in the neurotoxic actions of MDMA-like drugs.

However, the exact dopaminergic pharmacological action induced by these drugs that is involved in the neurotoxic actions has not been clearly elucidated. Since it is known that MDMA is a moderately active releaser of nonvesicular DA (8, 13, 14, 41), it has been commonly hypothesized that drug-induced DA release is responsible for the long-term effect [see for example (41)]. Yet, amphetamines are also known to inhibit monoamine oxidase (MAO) and inhibit the reuptake of DA (5, 14, 26, 34, 41, 44). Therefore, it is possible that one or both of these actions is also important in the neurotoxic effects of MDMA-like drugs.

We have previously reported on a number of MDMA structural analogues with decreased neurotoxic liability following single high doses. This includes the rigid analogue 5,6-methylenedioxy-2-aminoindan (MDAI; Fig. 1) that was devoid of any long-term effects on the serotonergic system after a single dose up to 40 mg/kg (30). However, MDAI does appear to be "MDMA-like" both behaviorally and pharmacologically. For instance, MDAI will substitute in rats trained to discriminate saline from MDMA (32) and in rats trained to discriminate saline from S-N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (33), the α-ethyl homologue of MDMA. Furthermore, like MDMA (8, 13, 14, 41, 44), MDAI is a potent releaser and uptake inhibitor of 5-HT in vitro (14). However, it should be noted that MDMA is a more potent releaser of nonvesicular DA from synaptosomes than is MDAI (14). Therefore, MDAI appears to be MDMA-like in an animal model and to have similar monoamine-releasing properties but with greater selectivity for 5-HT. However, MDAI does not seem to have the neurotoxic liability seen with MDMA.

The discovery of MDMA analogues with decreased neurotoxic potential provided a unique opportunity to attempt to induce a neurotoxic response by combining certain pharmacological actions with that of the nontoxic analogue. Therefore, the present study attempted to examine the relative importance of various dopaminergic actions by combining certain drug pretreatments with MDAI in an attempt to induce an MDMA-like 5-HT neurotoxic response.

METHOD

Materials

Fluoxetine was graciously provided by Eli Lilly Laboratories (Indianapolis, IN). S-(+)-Amphetamine sulfate and the HPLC standards were purchased from Sigma Chemical Company (St. Louis, MO). Clorgyline HCl, (−)-deprenyl HCl and GBR-12909 2HCl were purchased from Research Biochemicals Incorporated (Natick, MA). [^{3}H]Paroxetine was purchased from New England Nuclear (Boston, MA) at a specific activity of 28.8 Ci/mmol. 5,6-Methylenedioxy-2-aminoindan HCl was synthesized as described in Nichols et al. (30).

HPLC With Electrochemical Detection

Tissue samples were prepared by homogenizing the weighed brain areas from one hemisphere in 0.5 ml of 0.4 N HClO$_4$

containing 0.05% Na$_2$EDTA, and 0.1% Na$_2$S$_2$O$_5$, using a motor-driven Teflon pestle and Eppendorf 1.5-ml centrifuge tubes. An internal standard of 3-(3,4-dihydroxyphenyl)propionic acid was added. The samples were centrifuged at 14,000 × g for 4 min with a tabletop centrifuge. The supernatant was collected, and 50 μl was injected onto the HPLC column.

The HPLC-EC system consisted of the following equipment: a refrigerated autosampler (TosoHaas; Philadelphia, PA), a model 400 EG&G Princeton electrochemical detector (Princeton, NJ), and a Brownlee C18 reverse-phase analytical cartridge column (Anspec; Ann Arbor, MI). A series dual electrode cell set at E$_1$ = −200 mV and E$_2$ = 850 mV versus the Ag/AgCl reference electrode was used. A guard potential of Eg = 60 mV was also utilized. The mobile phase consisted of 50 mM NaH$_2$PO$_4$, 30 mM citric acid, 0.1 mM Na$_2$EDTA, 0.034% sodium octyl sulfate and 25% methanol. A flow rate of 1 ml/min gave approximately the following retention times: DOPAC, 6.10; 5-HIAA, 8.50; DA, 10.80; HVA, 12.80; and 5-HT, 24.10 min, respectively. The levels of monoamines and their metabolites were determined from standard curves using an Apple Macintosh SE computer with the Dynamax Methods Manager software (Rainin; Woburn, MA).

[^{3}H]Paroxetine Receptor Binding

A modified procedure of Marcusson et al. (23) was employed. A buffer consisting of 50 mM Tris HCl with 120 mM NaCl and 5 mM KCl (TNK) at pH 7.4 was utilized both for incubations and for tissue homogenization. Tissue samples were weighed and homogenized in 5 ml of TNK with a Brinkman polytron (setting 6, 2×20 seconds). The homogenates were centrifuged twice at 30,000 × g for 10 min with an intermittent wash, and the pellet was resuspended in the same volume of TNK.

Since it has previously been reported that only the B$_{max}$ and not the K$_D$ values are altered after MDMA treatment (4,30), it is possible to measure the amount of 5-HT uptake carrier with a single saturating (1 nM) concentration of [^{3}H]paroxetine (3,17). Specific binding was defined with 1 μM fluoxetine. Incubations were started by adding 150 μl of tissue homogenate to each tube to give a final volume of 1.65 ml. Tubes were allowed to equilibrate at 24°C for 1 h before adding 4 ml of ice-cold buffer and filtering through Whatman GF/C filters using a Brandel Cell Harvestor (Gaithersburg, MD). The tubes were washed twice with ice-cold buffer and the filters allowed to air dry. Filters were then placed into 20-ml plastic vials, and 10 ml of scintillation counting fluid was added. The vials were sealed and allowed to sit overnight before counting at an efficiency of 54%.

Statistical Analysis

Values are the mean ± S.E.M. for 6 to 8 animals in each treatment group. Separate saline controls were run in parallel with each experiment. All comparisons utilized an analysis of variance followed by a modified t-test post hoc comparison (ANOVA) as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX).

RESULTS

Monoamine Levels at 3 h After MDMA and MDAI

As seen in Table 1, monoamine levels were greatly affected at 3 h after a single dose of either MDMA or MDAI. After doses (40 mg/kg, SC) known to be neurotoxic for MDMA but not MDAI (30), both drugs substantially decreased the levels of

TABLE 1

MONOAMINE LEVELS 3 HOURS AFTER MDMA OR MDAI

Drug	Percent Control (pg/mg wet wt.)*				
	5-HT	5-HIAA	DA	DOPAC	HVA
Frontal Cortex:					
Saline	100 ± 10	100 ± 6	100 ± 9	100 ± 8	100 ± 8
	(380 ± 38)	(424 ± 25)	(52 ± 4)	(36 ± 3)	(47 ± 4)
MDMA	18 ± 2†	52 ± 3†	206 ± 16†	57 ± 11†	113 ± 9
(40 mg/kg)					
MDAI	7 ± 1†	50 ± 3†	147 ± 18†	134 ± 13†	163 ± 29†
(40 mg/kg)					
Striatum:					
Saline	100 ± 6	100 ± 5	100 ± 3	100 ± 5	100 ± 5
	(579 ± 32)	(935 ± 50)	(12,913 ± 451)	(1385 ± 75)	(807 ± 41)
MDMA	36 ± 4†	76 ± 4†	141 ± 6†	39 ± 3†	93 ± 8
MDAI	11 ± 1†	78 ± 3†	127 ± 5†	100 ± 6	213 ± 18†
Hippocampus:					
Saline	100 ± 2	100 ± 6	—‡	—	—
	(488 ± 12)	(651 ± 41)			
MDMA	13 ± 2†	75 ± 5†	—	—	—
MDAI	21 ± 3†	64 ± 3†	—	—	—

*Values are represented as the mean ± S.E.M. for an N = 6.
†Indicates significantly different from saline control ($p < 0.05$, ANOVA).
‡Below the level of detection.
Rats were injected subcutaneously with either saline, MDMA or MDAI and sacrificed 3 h after the last dose. Monoamine levels were determined by HPLC-EC as described in the Method section.

5-HT and its metabolite 5-HIAA in the frontal cortex, hippocampus and striatum. It is worth noting that 8 h after dosing, the levels of 5-HT in MDAI-treated animals had returned to approximately 80% of control, while the levels of 5-HT after MDMA were still depleted to 30% of control values (data not shown). Both drugs dramatically increased DA levels in the cortex and striatum. However, while MDMA caused a decrease in striatal and cortical DOPAC, with no changes in HVA, at non-neurotoxic doses, MDAI caused an increase in cortical DOPAC and HVA, and striatal HVA.

Monoamine Levels After MDAI With Dopaminergic Agents

Striatal monoamine levels 3 h after a combination of either clorgyline, deprenyl, GBR-12909 or S-amphetamine with MDAI are given in Table 2. The results in the hippocampus and striatum were similar to those reported for the frontal cortex. It is clear from the data that both the MAO$_A$ inhibitor clorgyline (2.5 mg/kg, IP) and the MAO$_B$ inhibitor deprenyl (10 mg/kg, IP) resulted in significant inhibition of DA metabolism, as indicated by the decreased levels of DOPAC and HVA 3 h after dosing. As anticipated, based on the selectivity of the isoenzymes for 5-HT, clorgyline but not deprenyl also appears to have inhibited the metabolism of 5-HT, as indicated by the decreased levels of 5-HIAA after clorgyline-saline treatment. Pretreatment with either clorgyline or deprenyl before MDAI (20 mg/kg, SC) resulted in a dopaminergic profile similar to that seen after MDMA. More specifically, the combination produced increases in DA and decreases in DA metabolites, as contrasted with the increases in both DA and its metabolites seen with MDAI alone. However, the combination of clorgyline with MDAI resulted in substantially higher levels of 5-HT than with either MDMA or MDAI alone. The monoamine profile after deprenyl plus MDAI gave the closest similarity to that seen after MDMA alone.

However, neither the clorgyline nor the deprenyl pretreatments before MDAI resulted in any significant decrease in the levels of 5-HT at 1 week after dosing (data not shown).

GBR-12909 (15 mg/kg, IP) appeared to have no effect on the monoamine profile seen 3 h after MDAI (30 mg/kg, SC). Therefore, it was not surprising to find that the combination of GBR-12909 with MDAI did not cause any changes in monoamine levels at 1 week (data not shown).

Pretreatment with S-amphetamine (5 mg/kg, IP) before MDAI (30 mg/kg, SC) did result in a monoamine profile at 3 h similar to that seen after MDMA alone, namely, increases in DA with decreases in DOPAC, 5-HT, and 5-HIAA. However, at 1 week, the levels of 5-HT and 5-HIAA were not significantly decreased in any of the three brain areas (data not shown). A significant long-term decrease in striatal DOPAC (to 70% of control) was seen following the combination of S-amphetamine and MDAI but not with either drug alone (data not shown).

Posttreatment of S-Amphetamine After MDAI

Previous work has indicated a possible dopaminergic component of the behavioral actions of MDMA at approximately 2 h after treatment (35,36). Since only the combination of S-amphetamine with MDAI resulted in any long-term change in a monoamine marker, the effect of posttreatment of S-amphetamine with MDAI was examined. As seen in Fig. 2, this drug regimen resulted in slight but significant decreases in the levels of 5-HT and 5-HIAA in all three brain areas examined. However, there was no significant decrease in the number of 5-HT uptake sites defined by [³H]paroxetine binding.

Subacute Combined Dosing With S-Amphetamine and MDAI

In an initial experiment, rats were injected (SC) with either 20 mg/kg MDAI or saline every 12 h for 2 days. At 15 min

TABLE 2

STRIATAL MONOAMINE LEVELS AT 3 H

Pretreatment†	Posttreatment	Percent Control (pg/mg wet wt.)*				
		5-HT	5-HIAA	DA	DOPAC	HVA
Saline	Saline	100 ± 11	100 ± 8	100 ± 3	100 ± 5	100 ± 8
		(505 ± 55)	(726 ± 57)	(16,792 ± 487)	(1343 ± 72)	(1021 ± 86)
Clorgyline (2.5 mg/kg)	Saline	143 ± 16‡	54 ± 1‡	128 ± 7‡	11 ± 2‡	14 ± 2‡
Deprenyl (10 mg/kg)	Saline	97 ± 10	100 ± 5	133 ± 6‡	42 ± 4‡	54 ± 5‡
Saline	MDAI (20 mg/kg)	12 ± 1‡	56 ± 11‡	134 ± 14‡	149 ± 17‡	246 ± 30‡
Clorgyline	MDAI	130 ± 10§	38 ± 7‡	205 ± 17‡§	7 ± 2‡§	27 ± 8‡§
Deprenyl	MDAI	17 ± 3‡	64 ± 8‡	154 ± 8‡	58 ± 3‡§	168 ± 25‡§
Saline	Saline	100 ± 6	100 ± 3	100 ± 3	100 ± 9	100 ± 3
		(350 ± 22)	(753 ± 24)	(17,300 ± 483)	(1456 ± 135)	(1356 ± 38)
GBR-12909 (15 mg/kg)	Saline	112 ± 6	95 ± 7	100 ± 2	88 ± 11	91 ± 6
S-Amph. (5 mg/kg)	Saline	100 ± 10	105 ± 9	121 ± 6‡	94 ± 4	130 ± 7‡
Saline	MDAI (30 mg/kg)	11 ± 3‡	69 ± 5‡	137 ± 7‡	107 ± 14	132 ± 4‡
GBR-12909	MDAI	7 ± 3‡	76 ± 8‡	120 ± 7‡	85 ± 6	148 ± 1*‡
S-Amph.	MDAI	6 ± 3‡	77 ± 6‡	156 ± 16‡	54 ± 15‡§	109 ± 3§

*Values are represented as the mean ± S.E.M. for an N = 6.
†Drugs were given 15 min before either a saline or MDAI treatment as indicated.
‡Indicates significantly different from saline control ($p < 0.05$, ANOVA).
§Indicates significantly different from saline/MDAI treatment ($p < 0.05$, ANOVA).

Rats were injected intraperitoneally with the indicated pretreatment drug 15 min before a subcutaneous injection of saline or MDAI. Animals were sacrificed 3 h after the last dose, and monoamine levels were determined by HPLC-EC as described in the Method section. The results in the frontal cortex and hippocampus are similar to those reported here.

before and 2 h after each of these doses, S-amphetamine (5 mg/ kg, IP) or saline was injected. In the S-amphetamine and MDAI treatment group, 50% of the animals died after 2 to 4 doses. One week after the last dose, an analysis of monoamine levels and [³H]paroxetine binding indicated a statistically significant ($p < 0.05$, ANOVA) 20 to 30% decrease in serotonergic parameters in the cortex, hippocampus and striatum of animals treated with MDAI alone (data not shown). These same serotonergic markers were significantly decreased with the combination of S-amphetamine and MDAI. In the case of hippocampal 5-HIAA and striatal 5-HT, as well as the numbers of cortical and striatal uptake sites, the decreases seen after the combination of S-amphetamine and MDAI were significantly greater than the decreases seen with MDAI alone (data not shown).

In a second study, the doses of both MDAI and S-amphetamine were decreased by a factor of two (10 mg/kg and 2.5 mg/ kg, respectively), and the dosing was repeated as above every 12 h for 4 days. As seen in Fig. 3, with this dosing regimen, MDAI alone did not result in a significant decrease in any serotonergic marker 1 week after the last dose. However, when MDAI was combined with S-amphetamine, significant decreases in serotonergic parameters were observed.
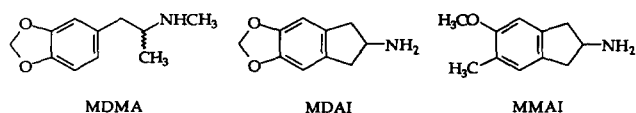

FIG. 1. Structures of MDMA analogues.

DISCUSSION

It is clear from the data in Table 1 that the effect of MDMA and MDAI on DA metabolites is very different. It has been noted before that, at neurotoxic doses, MDMA causes significant increases in DA levels with deceases in DOPAC 3 h after dosing (18, 25, 41, 48). Therefore, MDMA results in a transient decrease in the DOPAC/DA ratio (25) in frontal cortex and striatum. This is in stark contrast to the dopaminergic effects of MDAI at nonneurotoxic doses. At 3 h after MDAI, there are not only increases in the levels of DA but also increases in the levels of DOPAC and HVA. This short-term effect of MDAI is similar to that observed for the nonneurotoxic MDMA analogues, 3-methoxy-4-methylamphetamine and 5-methoxy-6-methyl-2-aminoindan (MMAI) (15). Therefore, while MDMA decreases the DOPAC/DA ratio, at nonneurotoxic doses, MDAI increases or has no effect on this measure of dopaminergic action.

The DOPAC/DA ratio can be altered by a number of agents, including: MAO inhibitors, DA uptake inhibitors, and nonvesicular DA releasers. In the latter case, it should be noted that Ask and co-workers (1,2) have recently shown that many nonvesicular releasers are somewhat selective for inhibition of MAO within neuron terminals as opposed to that outside the terminals. Therefore, if this DOPAC/DA ratio was a key indicator of the neurotoxic effects of MDMA, treatment with agents that decrease this ratio, in combination with a nonneurotoxic MDMA analogue such as MDAI, should result in a short-term monoamine profile similar to that seen with MDMA. Furthermore, the combination of one of these pharmacological agents with MDAI might result in long-term changes in serotonergic markers.
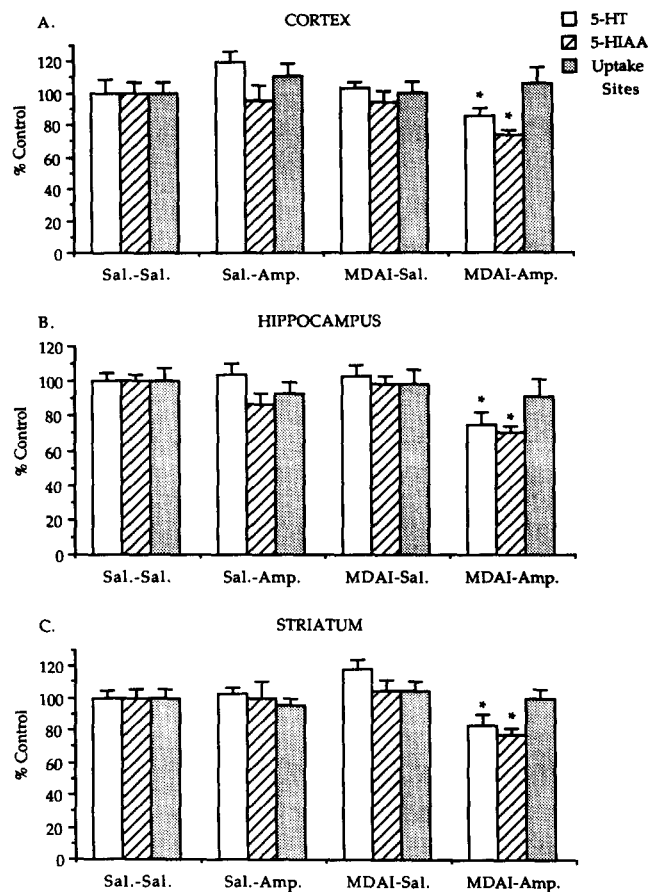
FIG. 2. Serotonergic markers at one week after MDAI and a posttreatment with S-amphetamine. Saline or 20 mg/kg MDAI was injected subcutaneously, followed at 2.5 and 5 h by either saline or 5 mg/kg of S-amphetamine (IP). Animals were sacrificed one week later. The levels of catecholamines were unchanged with drug treatments. The number of uptake sites was determined from [^{3}H]paroxetine binding as described in the Method section. Values are presented as the mean ± SEM for N = 6. Saline control values for frontal cortex (A) were: 5-HT, 353 ± 29; 5-HIAA, 419 ± 28 pg/mg wet wt.; and uptake, 19 ± 1 fmol/g wet wt. Saline control values for hippocampus (B) were: 5-HT, 278 ± 12; 5-HIAA, 682 ± 26 pg/mg wet wt.; and uptake, 16 ± 1 fmol/g wet wt. Saline control values for striatum (C) were: 5-HT, 417 ± 20; 5-HIAA, 929 ± 52 pg/mg wet wt.; and uptake, 22 ± 1 fmol/g wet wt. *Indicates significantly different from saline control value ($p<0.05$, ANOVA).
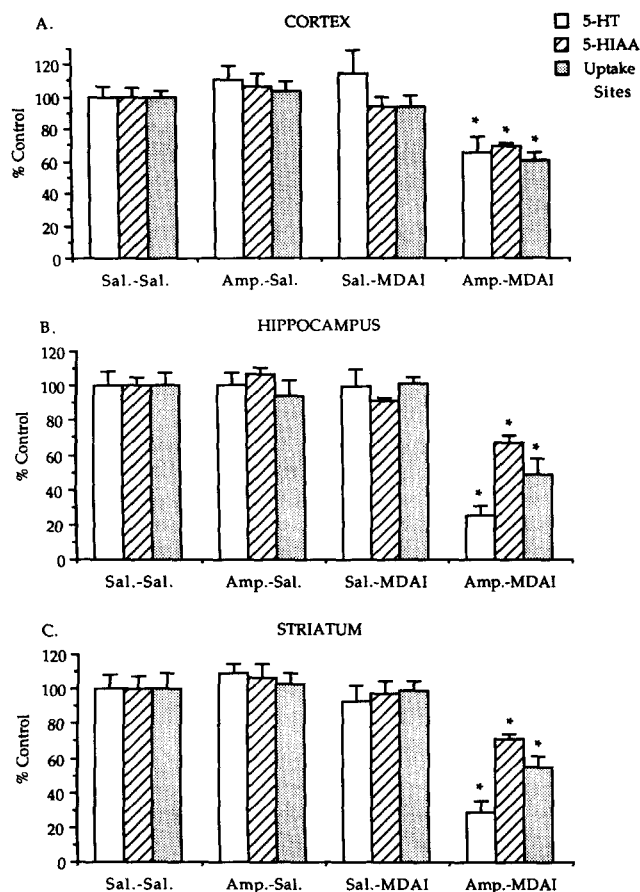
FIG. 3. Serotonergic markers one week after combined S-amphetamine and MDAI administration. Saline or 10 mg/kg MDAI was injected subcutaneously. At 15 min before and 2 h after MDAI or saline, either saline or 2.5 mg/kg S-amphetamine (IP) was injected. This was repeated every 12 h for 4 days. Animals were sacrificed one week after the last dose. Catecholamine levels were unchanged after drug treatments. The number of uptake sites was determined from [^{3}H]paroxetine binding as described in the Method section. Values are presented as the mean ± SEM for N = 6. Saline control values for frontal cortex (A) were: 5-HT, 377 ± 23; 5-HIAA, 664 ± 38 pg/mg wet wt.; and uptake, 18 ± 1 fmol/g wet wt. Saline control values for hippocampus (B) were: 5-HT, 471 ± 41; 5-HIAA, 1039 ± 49 pg/mg wet wt.; and uptake, 16 ± 1 fmol/g wet wt. Saline control values for striatum (C) were: 5-HT, 672 ± 55; 5-HIAA, 1253 ± 97 pg/mg wet wt.; and uptake, 19 ± 2 fmol/g wet wt. *Indicates significantly different from saline control value ($p<0.05$, ANOVA).

As seen in the present study, this was not found when the MAO$_A$ inhibitor clorgyline or the DA uptake inhibitor GBR-12909 was combined with MDAI. The monoamine profile was substantially different from that seen after MDMA alone (Table 2). More importantly, there were no signs of a neurotoxic response at 1 week (data not shown). When the MAO$_B$ inhibitor deprenyl was combined with MDAI, the levels of DA were increased at 3 h with decreases in DA metabolites, similar to the effect of MDMA. However, there were no long-term effects of this drug combination. This was also true when a multiple-dosing regimen was utilized. When deprenyl (10 mg/kg, IP) was followed by MDAI (20 mg/kg, SC every 12 h for 2 days) and the animals were sacrificed 1 week after the last dose, deprenyl failed to alter the long-term changes seen with MDAI alone. Therefore, it appears that the inhibition of either MAO$_A$, MAO$_B$ or DA uptake does not induce a neurotoxic response when com-

bined with a nonneurotoxic MDMA-like analogue.

In contrast to this, the present results do implicate nonvesicular DA release in the mechanism of MDMA neurotoxicity. First, it was found that the combination of the 5-HT releaser MDAI and the DA releaser S-amphetamine resulted in a monoamine profile at 3 h similar to that seen with MDMA alone. More specifically, this drug combination resulted in increased DA with decreases in 5-HT, 5-HIAA, and DOPAC with no change in HVA levels (Table 2). It is interesting to note that these changes in DA metabolites were only seen when the serotonergic agent was combined with the dopaminergic agent. Specifically, while the levels of DOPAC were unchanged and the levels of HVA increased after either S-amphetamine or MDAI alone, when combined, these drugs resulted in decreases in DOPAC and no changes in HVA. Therefore, it may be hypoth-

esized that the decreases in DOPAC are due not only to a DA- but also a 5-HT releasing action.

More significant, however, are the long-term effects of this drug combination. For instance, when S-amphetamine was given as a pretreatment to MDAI, a long-term decrease in striatal DA was seen. This is similar to the effect of MDMA at neurotoxic doses (41,48). Furthermore, when S-amphetamine is given as a posttreatment to MDAI, significant long-term decreases in 5-HT and 5-HIAA are noted in all three brain areas examined. However, the most marked effects are seen with a subacute dosing regimen.

As seen in Fig. 2, subacute dosing with either 10 mg/kg MDAI or 2.5 mg/kg S-amphetamine did not result in significant changes in any monoamine markers. However, when these drugs were combined, there was an approximately 40% decrease in serotonergic markers with no change in catecholamines. Since this is a decrease in not only the levels of 5-HT and 5-HIAA but also the number of 5-HT uptake sites that remain 1 week after dosing, as seen with other neurotoxic amphetamines, the most plausible explanation is a serotonergic neurotoxic response. Therefore, S-amphetamine is the only dopaminergic agent examined so far that potentiates the neurotoxic effects of MDAI. This is, however, similar to the L-DOPA potentiation of long-term effects of MDMA that has been previously reported by Schmidt and co-workers (42). There, it was reported that when L-DOPA and a peripheral decarboxylase inhibitor were combined with MDMA, a greater long-term effect on serotonergic markers was seen than with MDMA alone.

Caution should be used in interpreting the present results, since subacute treatment with higher doses (20 mg/kg) of MDAI alone does produce long-term changes in serotonergic parameters (data not shown). Therefore, one cannot conclude that S-amphetamine "induced" a neurotoxic response in MDAI-treated animals. However, in a separate study, we found that S-amphetamine did result in a serotonergic neurotoxic response when combined, using subacute dosing, with MMAI (Fig. 1), an MDMA analogue that is not neurotoxic even at high subacute doses (20).

It is interesting to note that approximately the same degree of neurotoxicity was induced by MDAI with S-amphetamine in all three brain areas (Fig. 3). A similar effect was seen when S-amphetamine was combined with MMAI (20). This occurred despite the differences in dopaminergic innervation that occur within the different brain areas (striatum >> frontal cortex > hippocampus). Therefore, even in the hippocampus, where the levels of DA are well below detection limits, S-amphetamine was still able to induce serotonergic neurotoxicity after MDAI. This might suggest that there is a link between the toxicity induced in the hippocampus and that seen in brain regions where the dopamine levels are higher, such as the striatum. The nature

of this link is unclear at this time, although it has been noted that 6-hydroxydopamine lesions in the striatum are able to block MDMA-induced serotonergic neurotoxicity not only in the striatum but also in the hippocampus and frontal cortex (40).

Alternatively, it is possible that S-amphetamine is inducing neurotoxicity in the hippocampus by releasing NE. However, results from much of the earlier work seem to argue against this possibility. For example, the enantiomers of MDA and MDMA show a stereoselectivity for DA uptake inhibition (44) and neurotoxic potency (13, 37, 38, 41), but only a small difference in enantiomeric activity is seen for inhibition of NE uptake (44). Selective lesioning of DA axons with bilateral injection of 6-hydroxydopamine in the substantia nigra or pretreatment with the selective DA uptake inhibitor GBR-12909 are also able to block MDMA neurotoxicity (40,47).

Furthermore, the short-term dramatic changes in DA, DOPAC and HVA levels seen after high doses of MDMA, while the levels of NE remain unchanged, would argue for a more important role of DA over NE in the effects of MDMA (18, 41, 48). This is further supported by the fact that certain agents, such as fluoxetine and 5-HT_2 antagonists, block not only the neurotoxic effects of MDMA but also the short-term changes in DA induced by MDMA (6, 29, 39). For instance, it has been reported that 5-HT_2 antagonists block or substantially attenuate the rise in L-DOPA utilization (29) and the increased levels of DA seen shortly after MDMA treatments (29,39). Interestingly, 5-HT_2 antagonists have also been shown to block the long-term reductions in serotonergic markers associated with high doses of MDMA (29, 39, 40). Therefore, not only are there significant changes in the levels of DA and its metabolites with no changes in the noradrenergic system with MDMA, but these responses in the dopaminergic system are effected by the same drugs, 5-HT uptake inhibitors and 5-HT_2 antagonists, that have been shown to block the drug-induced neurotoxicity. This suggests a more important role for DA over NE in the neurotoxic effect of MDMA.

In conclusion, these data clearly indicate that potentiation of a serotonergic neurotoxic response is possible by combining S-amphetamine with a relatively nonneurotoxic analogue of MDMA, which is a potent serotonin-releasing agent. This supports the hypothesis that DA, and more specifically, nonvesicular DA release, play a critical role in the serotonergic neurotoxicity of some MDMA-like drugs. Furthermore, the induction of a neurotoxic response in the hippocampus, a brain area with very little dopaminergic innervation, may imply a link between the drug effects in this and other brain areas.

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