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Effect of Acute Monoamine Depletion on 3,4-Methylenedioxymethamphetamine-Induced Neurotoxicity

JESSE BRODKIN, ANNA MALYALA AND J. FRANK NASH[1]

Departments of Psychiatry and Neuroscience, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-5000

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BRODKIN, J., A. MALYALA AND J. F. NASH. *Effect of acute monoamine depletion on 3,4-methylenedioxymethamphetamine-induced neurotoxicity.* PHARMACOL BIOCHEM BEHAV **45**(3) 647–653, 1993.—The effect of acute, reversible depletion of either serotonin [5-hydroxytryptamine (5-HT)] or dopamine (DA) on the long-term (7-day) decrease of brain 5-HT content produced after 3,4-methylenedioxymethamphetamine (MDMA) administration was investigated. The tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (α-MPT) significantly attenuated the acute increase in DA efflux produced by MDMA in the striatum as measured by in vivo microdialysis. Treatment with α-MPT had no effect on MDMA-induced 5-HT release. α-MPT treatment blocked the long-term (7-day) depletion of striatal 5-HT content after MDMA administration. The tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) completely blocked the acute increase in the extracellular concentration of 5-HT produced by MDMA. Although PCPA significantly attenuated the increase in DA efflux produced by MDMA, the effect was small in magnitude. More importantly, treatment with PCPA had no effect on MDMA-induced decrease of 5-HT uptake sites in the frontal cortex. These data are suggestive that acute depletion of DA but not 5-HT protects against the long-term neurotoxic effects of MDMA on 5-HT axon terminals. In addition, these data are supportive of the hypothesis that DA plays a major role in the neurotoxic effects of MDMA.

α-Methyl-*p*-tyrosine 3,4-Methylenedioxymethamphetamine Microdialysis Neurotoxicity *p*-Chlorophenylalanine

ACUTE, high-dose administration of 3,4-methylenedioxymethamphetamine (MDMA) and related compounds [e.g., methamphetamine, *p*-chloroamphetamine (PCA)] produces long-term decreases in the concentration of serotonin [5-hydroxytryptamine (5-HT)] and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in several forebrain regions of the rat (3,7,27,29,36). Although MDMA-induced damage to 5-HT axon terminals has been demonstrated with a variety of experimental methods, the mechanism by which MDMA produces this effect is poorly understood.

MDMA as well as PCA and methamphetamine increase the release of monoamines [i.e., 5-HT, dopamine (DA) and norepinephrine (NE)] with varying degrees of specificity (10, 17,20). The acute release of DA and/or 5-HT has been implicated in the long-term neurotoxic effects of amphetamine analogs including MDMA (1,35). For example, it has been hypothesized that an excessive and prolonged increase in DA neurotransmission plays a significant role in the long-term depletion of brain 5-HT content produced after MDMA administration (24,35). This is based upon the assumption that DA can be taken up into 5-HT axon terminals and converted to a neurotoxic intermediate such as 6-hydroxydopamine (6-OHDA) (33), hydrogen peroxide, quinones, or some free radical. This hypothesis is supported by pharmacological studies in which inhibition of either DA synthesis or reuptake blocks the long-term depletion of brain 5-HT produced after MDMA administration (30,35).

A similar hypothesis has been postulated to involve the serotonergic system. Excessive release of 5-HT could result in the formation of an endogenous neurotoxin such as 5,6-dihydroxytryptamine (5,6-DHT) (6) or tryptamine-4,5-dione (5,8), resulting in damage to the 5-HT axon terminal. In one study, administration of PCA was found to produce 5,6-DHT in a small number of animals (6). Similarly, acute depletion of 5-HT with *p*-cholorphenylalanine (PCPA) and reserpine has been reported to block the disappearance of 5-HT axon terminals in the frontal cortex after PCA administration (4).

The present study was designed to determine what effect acute, reversible depletion of either 5-HT or DA would have on the acute and long-term neurochemical changes produced by MDMA administration. If either DA, 5-HT, or both were involved in the long-term neurotoxic effect of MDMA, then

[1] Requests for reprints should be addressed to J. Frank Nash, Ph.D., The Procter & Gamble Company, Sharon Woods Technical Center, HB Building, Box 249, 11511 Reed Hartman Highway, Cincinnati, OH 45241-9974.

acute depletion of these monoamines should attenuate their release and block the long-term (7-day) damage to 5-HT axon terminals produced after MDMA administration. Consistent with other studies, acute depletion of DA blocked the long-term neurotoxic effect of MDMA on 5-HT axon terminals. In contrast, acute depletion of 5-HT had no effect on the loss of 5-HT uptake sites produced by MDMA. These data are supportive of the hypothesis that DA mediates the 5-HT-depleting effect of MDMA.

METHOD

Animals

Male Sprague-Dawley rats weighing 225–300 g were purchased from Zivic Miller Laboratory (Allison Park, PA) and used in all experiments. Animals were housed three per cage in a temperature-controlled room (23°C) with a 12 L : 12 D cycle (light on at 0600 h). Food and water were available ad lib.

Drugs

The racemic mixture of MDMA HCl was generously provided by Dr. David Nichols at Purdue University. Chloral hydrate, α-methyl-*p*-tyrosine (α-MPT) methyl ester, and PCPA methyl ester were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in saline and administered IP.

Experimental Procedure

In the first experiment, rats were anesthetized using chloral hydrate (400 mg/kg, IV) and placed in a stereotaxic frame. A concentric dialysis probe was implanted into the anterolateral striatum (A: 1.0, L: 3.0, V: −6.5, from bregma) according to the atlas of Paxinos and Watson (28). The probe was secured using a skull screw and dental cement and flushed with a modified Ringer's solution (in mM: NaCl 145, Na_2PO_4 6.0, KCl 1.7, KH_2PO_4 1.0, $CaCl_2$ 1.2 at pH 7.4). Animals were placed in clear plastic cages with food and water available ad lib.

On the following day, the dialysis probe was connected to an infusion pump set to deliver Ringer's solution at a rate of 1.8 μl/min. Each rat was perfused for 2 h prior to the initiation of each experiment. Following this initial perfusion, baseline samples were collected every 30 min over a 2-h time period. α-MPT (250 mg/kg) or vehicle was administered following the last baseline sample and 120 min before injection of MDMA (20 mg/kg) or vehicle. Dialysate samples were collected every 30 min for 5 h and the concentrations of DA and 5-HT were determined immediately.

In the second study, PCPA (150 mg/kg) or vehicle was administered on 3 consecutive days. The dialysis probe was implanted on the morning of the third PCPA injection. Twenty-four hours later, the dialysis experiment was begun as described in the preceding paragraph. Following a 2-h baseline, vehicle or MDMA (20 mg/kg) was administered and dialysate samples were collected every 30 min for 5 h.

Following termination of the dialysis studies, animals were returned to their home cages. One week later, animals were sacrificed and a 1-mm slice of the contralateral striatum at the level of the dialysis probe was removed and frozen in liquid nitrogen. The frontal cortex overlaying the striatum was dissected and frozen also. The concentrations of DA, 5-HT, and their metabolites were determined in the striatal tissue slices using previously reported procedures (21,22).

To determine the extent of acute monoamine depletion produced by α-MPT and PCPA, a separate group of animals was administered vehicle, α-MPT (250 mg/kg), or PCPA (150 mg/kg × 3 days) and sacrificed at 120 min or 24 h after the last injection, respectively. The concentrations of DA, 5-HT, and their metabolites were determined in the striatum using previously described high-performance liquid chromotography (HPLC) procedures (21,22).

Dialysis Probes

A concentric-shaped dialysis probe was constructed as described previously (37). The dialysis membrane extended 4.0 mm beyond the tip of the 26-ga stainless steel tubing. The percent recovery of these dialysis probes at room temperature was similar to previously published values (26).

Biochemical Measurements

The concentrations of DA and 5-HT were determined in dialysate samples using previously reported HPLC procedures (22,26). Briefly, each sample (50 μl) was injected onto a 3-μm C18 column (Phenomenex, Rancho Palos Verdes, CA) connected to an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) equipped with a glassy carbon electrode set at a potential of 0.75 V relative to the Ag/AgCl reference electrode. The mobile phase consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM disodium EDTA, 0.215 mM 1-octanesulfonic acid, 0.5% v/v triethylamine, and 5% v/v methanol delivered at a rate of 1.2 ml/min. The pH was adjusted to 4.25 with phosphoric acid. A Hewlett-Packard (HP 3396A) integrator was used to quantitate the concentrations of DA and 5-HT in the dialysate samples. Using these conditions, 5-HT and 3-methoxytyramine were separated completely.

[^{3}H]Paroxetine Binding

The number of 5-HT uptake sites was determined using a modification of previously published procedures (13,22). The frontal cortex was homogenized in 50 mM Tris-HCl (pH = 7.4 at 25°C) buffer containing 120 mM NaCl and 5 mM KCl using a polytron homogenizer at a setting of 7 for 10 s. This homogenate was then centrifuged at 40,000 × *g* for 10 min at 4°C. The process was repeated twice, and following the second centrifugation the pellet was resuspended in the Tris buffer (3 mg tissue wt/1.5 ml buffer) for binding studies. The homogenate (50 μl) was incubated with [^{3}H]paroxetine (DuPont NEN, Boston, MA, specific activity = 25 Ci/mM) in concentrations ranging from 0.005–1.0 nM at 22°C for 60 min in 1.4 ml Tris buffer. Incubations were done in the presence and absence of citalopram (1.0 μM) to determine specific binding. After incubation, the reaction was terminated by the addition of 4 ml Tris ice-cold buffer, which was rapidly filtered through Whatman GF/C filters (Whatman, Clifton, NJ). The filters were washed three times with 4 ml buffer. The filters were placed in liquid scintillation vials containing 10 ml Cytoscint (ICN Biomedical, Irvine, CA) and counted after overnight digestion. To obtain an estimate of the number of 5-HT uptake sites as measured by [^{3}H]paroxetine binding, a saturating concentration of [^{3}H]paroxetine (1 nM) was incubated with frontal cortical membranes using this procedure.

Statistical Analysis

The effect of α-MPT or PCPA on MDMA-induced increases in DA and 5-HT efflux in the striatum was statistic-

ally analyzed using a repeated-measures analysis of variance (ANOVA). The effect of PCPA or α-MPT on the decrease in [^{3}H]paroxetine binding in the frontal cortex produced by MDMA was analyzed using a two-way ANOVA. The effect of MDMA and vehicle on tissue content of DA, 5-HT, and their metabolites was analyzed using a one-way ANOVA. Comparisons between individual means were done by Scheffe's posthoc test. In all cases, treatment effects were considered statistically significant at $p < 0.05$.

RESULTS

The effect of α-MPT on MDMA-induced DA and 5-HT release in the striatum is illustrated in Figs. 1A and B. MDMA (20 mg/kg) produced a significant ($p < 0.001$) increase in the extracellular concentration of DA as compared to vehicle treatment (Fig. 1A). The extracellular concentration of DA remained significantly elevated for the duration of the experiment after MDMA administration. α-MPT (250 mg/kg) reduced the concentration of DA by approximately 50% in dialysate (i.e., 10 ± 1.6 to 5.7 ± 1.3 pg/50 μl) and postmortem striatal tissue samples (Table 1) 2 h after administration. Administration of α-MPT 120 min before MDMA significantly ($p < 0.001$) attenuated the increase in DA efflux produced by MDMA (Fig. 1A).

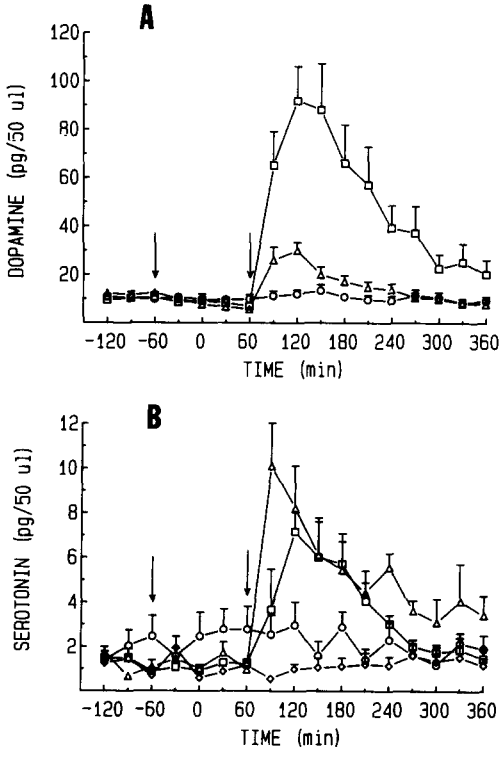

FIG. 1. Effect of α-methyl-*p*-tyrosine (α-MPT) on 3,4-methylenedioxymethamphetamine (MDMA)-induced dopamine (DA) (A) and 5-hydroxytryptamine (5-HT) (B) efflux in the striatum of awake, freely moving rats. α-MPT (250 mg/kg, IP) was administered 120 min before MDMA (20 mg/kg) as indicated by the arrows. Dialysate samples were collected every 30 min for 5 h. The treatment groups are represented by the following symbols: (□), vehicle + MDMA ($n = 5$), (△), α-MPT + MDMA ($n = 5$); (○), vehicle + vehicle ($n = 6$); and (◇), α-MPT + vehicle ($n = 4$). Each value represents the mean ± SE of n rats.

MDMA administration significantly ($p < 0.01$) increased the extracellular concentration of 5-HT in the striatum as compared to the vehicle control group (Fig. 1B). The maximum increase occurred between 30–60 min after administration and declined thereafter. Treatment with α-MPT had no effect on MDMA-induced 5-HT efflux (Fig. 1B) or tissue concentrations of 5-HT and 5-HIAA (Table 1).

The effect of PCPA (150 mg/kg × 3 days) on MDMA-induced DA release is illustrated in Fig. 2A. DA efflux after MDMA administration was attenuated significantly ($p < 0.05$) in PCPA-treated rats as compared to the vehicle/MDMA group. However, the magnitude of inhibition was much less than that observed in the α-MPT study (Fig. 1A vs. Fig. 2A). Treatment with PCPA had no effect on striatal concentrations of DA or its metabolites (Table 1).

Administration of PCPA (150 mg/kg × 3 days) significantly ($p < 0.01$) decreased tissue concentrations of 5-HT and 5-HIAA by approximately 75 and 85%, respectively (Table 1). The extracellular concentration of 5-HT was below the limit of detection (i.e., less than 0.5 pg/50 μl) in animals administered PCPA (Fig. 2B). Moreover, the increase in 5-HT efflux produced by MDMA in vehicle-treated rats was abolished completely in the PCPA-treated group challenged with MDMA (Fig. 2B).

The effect of α-MPT and PCPA on the concentrations of 5-HT and 5-HIAA in the striatum and the number of [^{3}H]paroxetine binding sites in the frontal cortex 7 days after MDMA administration is presented in Table 2 and Fig. 3, respectively. Consistent with previous studies (21,24,25), MDMA (20 mg/kg) significantly decreased the concentration of 5-HT and 5-HIAA in the striatum 7 days following administration as compared to vehicle-treated rats (Table 2). Likewise, the number of [^{3}H]paroxetine binding sites in the cortex was significantly ($p < 0.05$) reduced from 302 ± 13 fmol/mg protein in vehicle-treated rats to 237 ± 23 fmol/mg protein 7 days after MDMA administration (Fig. 3). Treatment with α-MPT completely blocked the decrease in 5-HT and 5-HIAA content (Table 2) and the number of 5-HT uptake sites (Fig. 3) produced by MDMA. The concentrations of 5-HT and 5-HIAA were significantly reduced 8 days after PCPA administration as compared to the vehicle control group (Table 2). However, consistent with the study of Dewar et al. (9), PCPA had no effect on the number of 5-HT uptake sites as measured by [^{3}H]paroxetine binding sites (Fig. 3). MDMA significantly ($p < 0.05$) decreased the concentration of 5-HT and 5-HIAA in PCPA-treated rats as compared to the group treated with PCPA and vehicle (Table 2). Moreover, treatment with PCPA had no effect on MDMA-induced decrease of [^{3}H]paroxetine binding in the cortex (Fig. 3).

DISCUSSION

Administration of MDMA increased the extracellular concentrations of DA and 5-HT in the striatum of awake, freely moving rats, consistent with several previous studies (12,14, 21,38). The acute depletion of brain DA content following inhibition of tyrosine hydroxylase by α-MPT significantly decreased MDMA-induced DA efflux in the striatum. Treatment with α-MPT had no effect on the acute release of 5-HT produced after MDMA administration. In contrast, treatment with PCPA completely blocked MDMA-induced 5-HT release. Although treatment with PCPA significantly inhibited MDMA-induced DA release, the effect was much less than that observed after α-MPT administration (Fig. 1B vs. Fig. 2B). Most importantly, α-MPT treatment completely blocked

TABLE 1

EFFECT OF α-MPT AND PCPA ON THE CONCENTRATION OF DA, 5-HT, AND THEIR METABOLITES IN THE STRIATUM

Treatment	n	Concentration (pg/μg protein)				
		DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle	6	93 ± 7	12 ± 2	11 ± 1	2.9 ± 0.2	6.1 ± 0.4
α-MPT	5	49 ± 3*	5 ± 1*	4 ± 1*	2.5 ± 0.4	5.3 ± 0.3
PCPA	5	87 ± 9	9 ± 1	7 ± 1	0.8 ± 0.1*	0.9 ± 0.3*

α-MPT (250 mg/kg) was administered 120 min before sacrifice. The last injection of PCPA (150 mg/kg × 3 days) was administered 24 h before rats were sacrificed. Each value represents the mean ± SE of n rats.

*Significant ($p < 0.05$) decrease as compared to vehicle-treated rats.

the long-term (7-day) depletion of striatal 5-HT content whereas PCPA treatment did not block either the decrease in striatal 5-HT content or the loss of [^{3}H]paroxetine binding sites in the frontal cortex produced by MDMA. These data are suggestive that acute depletion of DA but not 5-HT will block the long-term neurotoxic effects of MDMA on 5-HT axon terminals.

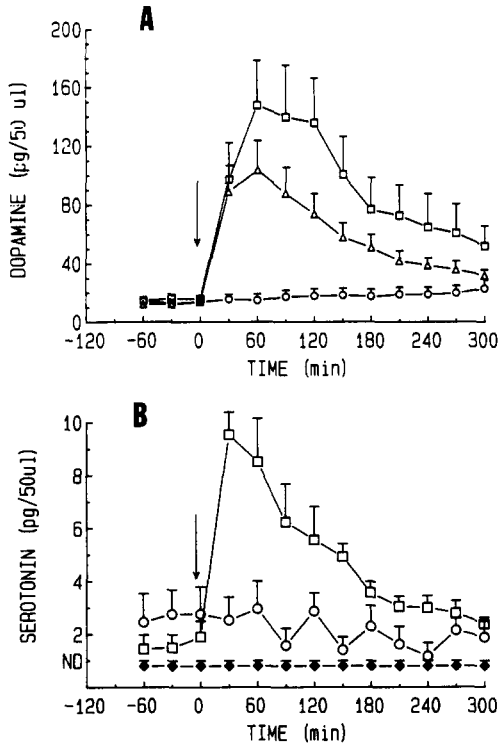

FIG. 2. Effect of p-chlorophenylalanine (PCPA) on 3,4-methylenedioxymethamphetamine (MDMA)-induced dopamine (DA) (A) and 5-hydroxytryptamine (5-HT) (B) efflux in the striatum of awake, freely moving rats. The last dose of PCPA (150 mg/kg, IP, × 3 days) was administered 24 h before administration of MDMA (20 mg/kg, IP). Dialysate samples were collected every 30 min for 5 h. The treatment groups are represented by the following symbols: (□), vehicle + MDMA (n = 4); (A: △; B: ♦), PCPA + MDMA (n = 7); (○), vehicle + vehicle (n = 6). Each value represents the mean ± SE of n rats. The concentration of 5-HT was nondetectable (ND) in rats treated with PCPA.

MDMA-induced DA release is mediated most likely via the DA uptake carrier. For example, DA uptake inhibitors attenuate the release of DA produced by local infusion of MDMA into the striatum (23). However, there is some evidence that is suggestive that 5-HT contributes to the stimulatory effect of MDMA on DA release (21,24,32). Several 5-$HT_{2/1C}$ receptor antagonists have been reported to inhibit MDMA-induced activation of tyrosine hydroxylase as well as the release of DA (21,24,32). In the present study, treatment with α-MPT did not completely eliminate MDMA-induced DA release. Interestingly, acute depletion of 5-HT after treatment with PCPA significantly inhibited the increase in the extracellular concentration of DA produced by MDMA administration. Thus, it is probable that MDMA-induced DA release occurs via the DA uptake carrier and by interacting with 5-$HT_{2/1C}$ receptors either directly or indirectly through the release of 5-HT. Regardless of the precise mechanism, it is clear that MDMA increases the extracellular concentration of DA, which remains elevated even 5 h following administration.

There are a number of studies that support the hypothesis that prolonged and excessive increases in DA neurotransmission play a role in the long-term 5-HT neurotoxicity produced by MDMA. First, treatment with DA uptake inhibitors such as GBR 12909 inhibit MDMA-induced DA release (23) and block the long-term depletion of brain 5-HT content (35). Second, depletion of DA using α-MPT (2,30,31,35) or lesions of the substantia nigra (30) blocks the 5-HT neurotoxic effects of MDMA as well as methamphetamine and PCA. The protective effect of substantia nigra lesions is not limited to the striatum; that is, forebrain regions such as the hippocampus and frontal cortex that are richly innervated by 5-HT but not DA axon terminals are protected from the neurotoxic effect of MDMA by substantia nigra lesions (30). Third, the protective effect of acute DA depletion can be reversed by administration of the DA precursor L-DOPA (30,35). Fourth, administration of 5-$HT_{2/1C}$ antagonists block the long-term depletion of brain 5-HT produced by MDMA as well as the acute activation of tyrosine hydroxylase and the release of DA (21,23, 24,32). Fifth, the amount of DA released acutely following administration of MDMA and related compounds (i.e., MDA, MBDB, MDE) correlates with the extent of 5-HT depletion 7 days later (25). Finally, the present study provides direct evidence that α-MPT inhibits the acute release in DA produced by MDMA administration. Collectively, these data are supportive of the hypothesis that DA is involved in the long-term depletion of 5-HT produced by MDMA administration.

If DA is neurotoxic to 5-HT axon terminals under appro-

TABLE 2

EFFECT OF α-MPT OR PCPA ON MDMA-INDUCED 5-HT AND 5-HIAA DEPLETION IN THE STRIATUM

Pretreatment	MDMA (20 mg/kg)	n	Concentration (pg/μg protein) 5-HT	5-HIAA
Vehicle	–	5	2.38 ± 0.27	5.38 ± 0.24
	+	7	1.19 ± 0.22*	3.64 ± 0.68*
α-MPT	–	3	2.61 ± 0.15	5.00 ± 0.30
	+	4	2.08 ± 0.15	5.81 ± 0.57
PCPA	–	4	1.58 ± 0.06*	2.66 ± 0.54*
	+	6	0.98 ± 0.13*†	1.48 ± 0.12*†

Each value represents the mean ± SE of n rats. The last injection of PCPA (150 mg/kg × 3 days) was 24 h before MDMA (20 mg/kg). α-MPT (250 mg/kg) was administered 120 min before MDMA. Rats were killed 7 days after administration of MDMA.

*Significant ($p < 0.05$) decrease as compared to the vehicle + vehicle treatment group.

†Significant ($p < 0.05$) decrease as compared to the PCPA + vehicle treatment group.

priate conditions, the question remains where and how does DA affect brain areas such as the hippocampus and frontal cortex that have little or no DA innervation. Because lesions of the substantia nigra block MDMA-induced 5-HT depletion in the hippocampus and frontal cortex (30), it is possible that there is limited diffusion of DA from areas such as the striatum to the hippocampus and/or frontal cortex. It is also possible that what little DA innervation known to exist in the hippocampus and cortex may be sufficient to produce a "neurotoxic" increase in DA under appropriate conditions. Alternatively, some DA may be released from noradrenergic terminals. However, these explanations are speculative. Clearly, these possibilities and others require further study.

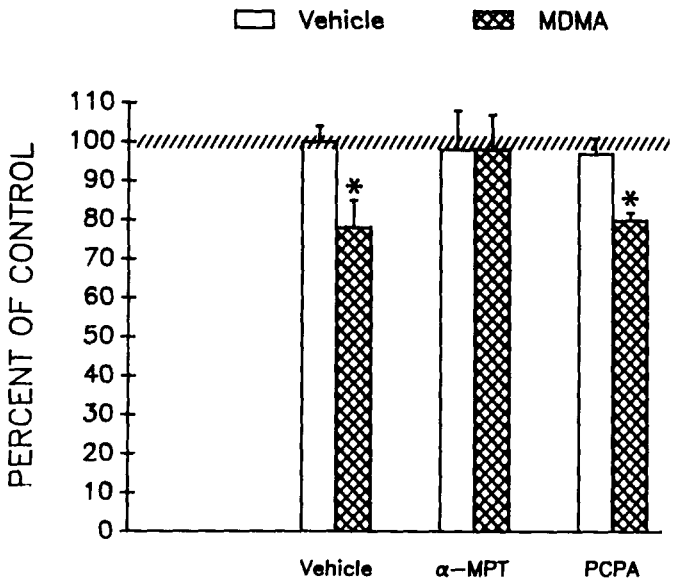

FIG. 3. Effect of α-methyl-p-tyrosine (α-MPT), p-chlorophenylalanine (PCPA) or vehicle on 3,4-methylenedioxymethamphetamine (MDMA)-induced decrease in [^{3}H]paroxetine ([^{3}H]PXT) binding in the frontal cortex 7 days following administration. Each value represents the mean ± SE of three to seven rats. Rats were sacrificed 7 days following administration of MDMA (20 mg/kg). The frontal cortex was frozen and the number of 5-hydroxytryptamine (5-HT) uptake sites was estimated using [^{3}H]PXT as described in the Method section. *Significant ($p < 0.05$) decrease as compared to vehicle-treated group.

The evidence is not as strong for the hypothesis that excessive activation of serotonergic pathways is responsible for the 5-HT neurotoxic effects of MDMA and related compounds. It is possible that 5-HT may be converted to a neurotoxic intermediate that produces damage to the 5-HT axon terminal (5,6,8). Because 5-HT uptake inhibitors are known to block the acute, reversible and long-term, irreversible decrease in 5-HT content produced by MDMA or PCA administration (11,29), it follows that uptake of these amphetamine analogs and/or release of 5-HT are important aspects related to their neurotoxic effects. However, in the present study depletion of brain 5-HT by 90% had no effect on the decrease in 5-HT uptake sites in the cortex produced by MDMA as assessed by [^{3}H]paroxetine binding. Similarly, PCPA treatment was found to have no effect on the depletion of brain 5-HT content produced by PCA (11). Thus, the failure of PCPA to protect against the 5-HT-depleting effect of MDMA is suggestive that the acute increase in 5-HT efflux is not responsible for the neurotoxic effect of MDMA. This finding is consistent with the observation that the acute (reversible) and long-term (irreversible) effects of MDMA and PCA are separable events. For example, MDMA increases the extracellular concentration of 5-HT within 30 min, followed by a return to baseline values within 3–4 h after administration [(12); Figs. 1B and 2B]. However, 5-HT uptake inhibitors such as fluoxetine block the depleting effect of MDMA even when administered 6 h after MDMA (29). This evidence does not infer that the 5-HT transporter has nothing to do with the 5-HT neurotoxicity but rather that the role of 5-HT itself or an oxidative derivative being involved in the neurotoxicity produced by MDMA or PCA is not supported by the majority of experimental evidence.

Although it has been suggested that MDMA or one of its metabolites may be neurotoxic, the data presented herein and

elsewhere do not support this hypothesis (15,16,18,19,34). Of the two primary metabolites of MDMA, 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymethamphetamine, only the latter has been found to be neurotoxic (16,34). However, 2,4,5-trihydroxymethamphetamine also has long-term effects on dopaminergic neurons, which, unlike MDMA, makes it a nonselective neurotoxin. If MDMA or one of its metabolites are responsible for the neurotoxicity, this would infer at least two explanations: a) that MDMA is only taken up by 5-HT axon terminals and/or b) that the enzymes responsible for this conversion are localized exclusively in 5-HT terminals. However, as presented in Figs. 1 and 2, MDMA releases both 5-HT and DA. Thus, it is likely that it is taken up into both 5-HT and DA axon terminals. The possibility that MDMA or one of its metabolites is converted selectively within the 5-HT axon terminals or only reaches neurotoxic concentrations within 5-HT terminals cannot be excluded. However, the pharmacological studies with DA uptake and synthesis inhibitors make this a somewhat remote possibility. Therefore, it is unlikely that MDMA itself or one of its metabolites mediates the neurotoxic effects of MDMA.

The data obtained in the present study are supportive of the hypothesis that DA plays a role in the neurotoxic effect of MDMA. Inhibition of the acute release of DA but not 5-HT blocked the long-term depleting effect of MDMA on 5-HT in the striatum. The precise role of DA in the neurotoxic effects of MDMA and related compounds remains to be determined.

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