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EFFECT OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE ON [^{3}H]PAROXETINE BINDING IN THE FRONTAL CORTEX AND BLOOD PLATELETS OF RATS

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Abstract—The effects of single or repeated administration of the racemic mixture of 3,4-methylenedioxymethamphetamine (MDMA; 20 mg/kg, s.c.) on the number (B_{max}) of serotonin (5-HT) uptake sites as determined by [^{3}H]paroxetine binding and the concentration of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were measured in the frontal cortex and blood platelets of rats 1 and 7 days following its administration. A single injection of MDMA significantly ($P < 0.05$) decreased the number of [^{3}H]paroxetine binding sites as well as the concentrations of 5-HT and 5-HIAA in the frontal cortex but not in platelets 7 days following administration. Repeated injections of MDMA (twice daily for 4 days) significantly ($P < 0.05$) decreased the number of 5-HT uptake sites and the concentration of 5-HT and 5-HIAA in the frontal cortex but not in platelets 7 days following administration. Pretreatment with the 5-HT$_2$/5-HT$_{1C}$ antagonist, ketanserin, inhibited the MDMA-induced decrease in 5-HT and 5-HIAA concentrations and the number of [^{3}H]paroxetine binding sites in the frontal cortex 7 days following a single administration. These data are suggestive that blood platelets are less sensitive than brain tissue to the 5-HT-depleting effects of MDMA. The ability of ketanserin pretreatment to block MDMA-induced decreases in [^{3}H]paroxetine binding sites in the frontal cortex is suggestive that 5-HT$_2$/5-HT$_{1C}$ receptors may be involved in the neurotoxic effects of MDMA.

Acute or subchronic administration of 3,4-methylenedioxymethamphetamine (MDMA) significantly decreases the concentration of serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in several brain regions of rodents as well as primates [1–3]. Immunocytochemical evidence indicates that MDMA selectively damages fine axon terminals which are believed to arise from cell bodies located in the dorsal raphé nuclei of the midbrain [4, 5]. The loss of axon terminals is supported by the finding that MDMA administration significantly decreases the number of 5-HT uptake sites in the brain, as identified by [^{3}H]paroxetine binding [6, 7].

Blood platelets have a 5-HT uptake carrier which resembles that found in brain tissue [8]. It has been suggested that the 5-HT uptake site on platelets may be a peripheral model of presynaptic 5-HT axon terminals [9]. Changes in the number of 5-HT uptake sites in the platelets of rats have been reported following chronic treatment with desmethylimipramine or electroconvulsive shock [10] and stressful stimuli [11]. In addition, a number of studies have found a decrease in the number of 5-HT uptake sites in the platelets of depressed patients as compared to normal volunteers, and this has been suggested to reflect diminished serotonergic function in depression [12]. If MDMA affected platelet measures in rodents, it would raise the possibility that such measures could provide an index of MDMA toxicity in humans.

Although MDMA-induced depletion of brain 5-HT content is well documented, the effect of MDMA on the concentration of 5-HT in other tissues has not been reported. The present study examined the effects of MDMA on 5-HT content and [^{3}H]paroxetine binding in platelets and brain tissue 1 and 7 days following single or repeated drug administration. In addition, the effect of pretreatment with ketanserin, a 5-HT$_2$/5-HT$_{1C}$ antagonist [13], on MDMA-induced decreases in 5-HT and 5-HIAA content as well as 5-HT uptake sites, as measured by [^{3}H]paroxetine binding in the frontal cortex, was determined on the basis of previous studies which found that ketanserin blocks the 5-HT-depleting effect of MDMA in the striatum [14].

MATERIALS AND METHODS

Animals. Male, Sprague–Dawley rats were purchased from Zivic Miller (Hillson, PA) and used in all experiments. Animals were housed six per cage in a temperature-controlled room (23°) with a 12/12 hr lighting schedule (lights on at 6:00 a.m.). Food and water were available *ad lib*.

Drugs. The racemic mixture of MDMA hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD). Ketanserin tartrate was obtained from Janssen Pharmaceutica (Beerse, Belgium). MDMA was dissolved in saline and administered s.c.; ketanserin was dissolved in 0.01 M tartaric acid and administered i.p.

Experimental procedures. The effects of MDMA

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(20 mg/kg, s.c.) or vehicle on [^{3}H]paroxetine binding and the concentrations of 5-HT and 5-HIAA were determined in platelet and frontal cortex membrane preparations. In the acute (MDMA 1×) experiments, rats were administered MDMA (20 mg/kg, s.c.) or vehicle at 8:00 a.m. and killed 1 and 7 days later. In the subchronic (MDMA 8×) study, rats were administered MDMA (20 mg/kg, s.c., twice daily for 4 days) or vehicle at approximately 7:30 a.m. and 6:00 p.m. and killed 1 and 7 days following the last drug injection. In another experiment, [^{3}H]paroxetine binding and the content of 5-HT and 5-HIAA were measured in the frontal cortex and platelets of rats that had received ketanserin (3 mg/kg, i.p.) 1 hr prior to the administration of MDMA (20 mg/kg) and were killed 7 days later.

Animals were decapitated, and trunk blood (5–7 mL) was collected in plastic tubes containing the anticoagulant, EDTA (1% in saline). Platelet-rich plasma was obtained following centrifugation (600 *g* for 2.5 min) at room temperature in a Sorvall GLC-2 centrifuge with a swinging bucket rotor. The total platelet population was isolated using previously described methods [15]. Platelet membranes were prepared by lysis and homogenization of the platelet pellet [16].

Immediately following decapitation, the brain was rapidly removed from each skull and placed on an ice-cold glass plate. The frontal cortex was dissected by hand and immediately frozen on dry ice. The samples were kept at −80° until the time of assay.

Assay for 5-HT and 5-HIAA in the frontal cortex and blood platelets. The concentrations of 5-HT and 5-HIAA in the frontal cortex were determined by HPLC with electrochemical detection as previously described [14]. Briefly, each sample was homogenized in 0.2 N perchloric acid/0.01% cysteine and spun at 9500 *g* for 20 min to obtain the supernatant. Fifty microliters of each sample was injected onto a C18 reverse phase column (Beckman, San Ramon, CA) connected to an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN). The HPLC mobile phase consisted of a 0.05 M citrate-phosphate buffer (pH 2.75) with 0.01 M disodium ethylenediamine tetraacetate, 0.042% (w/v) octyl sodium sulfate and 20% (v/v) methanol. The concentrations of 5-HT and 5-HIAA were determined by comparing peak heights of each sample with the corresponding standard peak height.

Platelet-rich plasma (0.5 mL) was spun (11,950 *g* for 10 min at 4°), and the resulting platelet pellet was reconstituted and lysed in 0.5 mL of 0.4 N perchloric acid/0.01% cysteine. The supernatant of each sample was then obtained following centrifugation (11,950 *g*, 10 min, 4°), and 50 μL was analyzed for 5-HT. The concentration of 5-HT was determined in blood platelets using the same analytical procedure described in the preceding paragraph.

[^{3}H]*Paroxetine binding.* The frontal cortex was homogenized in 50 mM Tris HCl (pH 7.4 at 25°) buffer containing 120 mM NaCl and 5 mM KCl using a polytron homogenizer at a setting of 7 for 10 sec. This homogenate was then centrifuged at 40,000 *g* for 10 min at 4°. The process was repeated twice, and following the second centrifugation, the pellet was resuspended in the Tris buffer (7 mg tissue wt/mL of buffer) for binding studies.

[^{3}H]Paroxetine binding in frontal cortical membranes was performed using the methods described by Habert *et al.* [17]. Briefly, 0.1 mL of homogenate was incubated with [^{3}H]paroxetine (DuPont NEN, sp. act. 27.9 Ci/nmol) in concentrations ranging from 0.03 to 2.5 nM, at 22° for 60 min in 1.0 mL of Tris buffer. Incubations were done in the presence and absence of fluoxetine (10 μM) to determine specific binding. After incubation, the reaction was terminated by the addition of 4 mL of ice-cold Tris buffer which was filtered rapidly through Whatman GF/C filters. The filters were washed three times with 4 mL buffer. The filters were placed in liquid scintillation vials containing 10 mL CytoScint™ (ICN Biomedical) 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 (2 nM) was incubated with frontal cortical membranes using this procedure.

[^{3}H]Paroxetine binding to platelet membranes was performed using the methods of Plenge and Mellerup [18]. Platelet membranes were incubated in six concentrations of [^{3}H]paroxetine (0.015 to 2.0 nM) in the presence and absence of fluoxetine (10 μM) to determine specific binding. Filtration and incubation times were the same as for the frontal cortex.

The tissue pellets were dissolved with 1 M NaOH, and the protein content was determined according to the method of Lowry *et al.* [19].

Statistical analysis. Scatchard analysis was used to calculate the dissociation constants (K_d) and the density (B_{max}) of [^{3}H]paroxetine binding sites in platelet and frontal cortex membrane preparations. One way analysis of variance was used to determine the overall significance of MDMA on the concentrations of 5-HT and 5-HIAA and the number of 5-HT uptake sites. Differences between individual treatment groups were determined using the Bonferroni *t*-test. In all cases, statistical significance was set at $P < 0.05$.

RESULTS

The effects of single or repeated administration of MDMA (20 mg/kg) on 5-HT content and on the B_{max} and K_d of [^{3}H]paroxetine binding in platelets 1 and 7 days following drug administration are presented in Table 1. Since no difference was found between the vehicle treatment groups, these values were pooled. A complete Scatchard analysis was used to calculate B_{max} and K_d of [^{3}H]paroxetine binding in the platelets obtained from each rat. The administration of MDMA had no significant effect on the number or affinity of 5-HT uptake sites as determined by [^{3}H]paroxetine binding. Repeated administration of MDMA significantly ($P < 0.05$) decreased the concentration of 5-HT in the platelets 24 hr following the last injection. No difference in platelet 5-HT content was found at any other time point in the MDMA-treated rats as compared to the vehicle control group.

The single or repeated injection of MDMA

Table 1. Effects of single or repeated injection of MDMA (20 mg/kg) on the B_{max} and K_d of [^{3}H]paroxetine binding and on the concentration of 5-HT in platelets 1 and 7 days following drug administration

Treatment	Post-treatment interval (days)	B_{max} (fmol/mg protein)	K_d (nM)	5-HT (ng/10^6 platelets)
Vehicle		4254 ± 261 (9)	0.069 ± 0.08 (9)	0.39 ± 0.04 (14)
MDMA (1×)	1	3686 ± 100 (3)	0.046 ± 0.02 (3)	0.25 ± 0.04 (6)
	7	4898 ± 674 (3)	0.089 ± 0.08 (3)	0.36 ± 0.03 (6)
MDMA (8×)	1	3715 ± 146 (3)	0.040 ± 0.083 (3)	0.16 ± 0.02* (5)
	7	4436 ± 97 (3)	0.053 ± 0.004 (3)	0.57 ± 0.06 (5)

Each value is the mean ± SE of the number in parentheses. MDMA (20 mg/kg, s.c.) was administered acutely (1×) or subchronically (8×) as described in Materials and Methods.
* Significantly ($P < 0.05$) different from vehicle-treated rats.

Table 2. Effect of single or repeated injection of MDMA (20 mg/kg) on the number (B_{max}) of [^{3}H]paroxetine binding sites and the concentration of 5-HT and 5-HIAA in the frontal cortex of rats 1 and 7 days following drug administration

Treatment	Post-treatment interval (days)	B_{max} (fmol/mg protein)	5-HT (ng/mg tissue)	5-HIAA (ng/mg tissue)
Vehicle		359 ± 11 (12)	0.27 ± 0.01 (28)	0.17 ± 0.01 (28)
MDMA (1×)	1	320 ± 22 (6)	0.18 ± 0.02* (9)	0.12 ± 0.01* (9)
	7	260 ± 36* (3)	0.12 ± 0.02* (6)	0.08 ± 0.01* (6)
MDMA (8×)	1	144 ± 6* (6)	0.08 ± 0.01* (10)	0.06 ± 0.01* (10)
	7	106 ± 6* (6)	0.07 ± 0.01* (9)	0.05 ± 0.01* (9)

Each value is the mean ± SE of the number in parentheses. MDMA (20 mg/kg, s.c.) was administered acutely (1×) or subchronically (8×) as described in Materials and Methods.
* Significantly ($P < 0.05$) different from vehicle-treated rats.

significantly ($P < 0.05$) decreased the concentration of 5-HT and 5-HIAA in the frontal cortex 1 and 7 days following administration as compared to vehicle-treated controls (Table 2). Repeated administration of MDMA significantly ($P < 0.01$) decreased the number of [^{3}H]paroxetine binding sites in the frontal cortex 1 and 7 days following the last injection. A single administration of MDMA significantly ($P < 0.05$) decreased the estimated number of 5-HT uptake sites at 7 days post-dosage (Table 2).

Figure 1 presents the effect of MDMA (20 mg/kg) on the number (B_{max}) of [^{3}H]paroxetine binding sites in the frontal cortex 7 days following a single administration. MDMA administration significantly ($P < 0.05$) reduced the B_{max} of [^{3}H]paroxetine binding sites compared to vehicle-treated controls. Pretreatment with the 5-HT_2/5-HT_{1C} antagonist ketanserin (3 mg/kg), 1 hr prior to MDMA administration, inhibited the MDMA-induced decrease in B_{max} of [^{3}H]paroxetine binding (Fig. 1) at this time point. Ketanserin alone had no effect on [^{3}H]paroxetine binding in the frontal cortex. No change was observed in the affinity constant (K_d) for [^{3}H]paroxetine in any of the treatment groups (data not shown).

The effects of MDMA with and without ketanserin pretreatment on the concentrations of 5-HT and 5-HIAA in the frontal cortex are presented in Table 3. MDMA (20 mg/kg) significantly ($P < 0.05$) decreased 5-HT and 5-HIAA content in the frontal cortex 7 days following a single administration. As

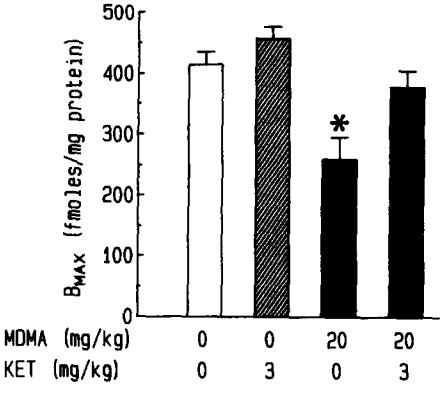

Fig. 1. Effect of ketanserin (KET) pretreatment on MDMA-induced decrease in [^{3}H]paroxetine binding (B_{max}) in the frontal cortex. Each value is the mean ± SE of three rats. Ketanserin (3 mg/kg, i.p.) was administered 1 hr prior to MDMA (20 mg/kg, s.c.). Rats were killed 7 days later. The asterisk (*) indicates a significant ($P < 0.05$) decrease as compared to the other treatment groups.

was the case for the number of [^{3}H]paroxetine binding sites, pretreatment with ketanserin significantly ($P < 0.05$) inhibited MDMA-induced 5-HT and 5-HIAA depletion in the frontal cortex. Ketanserin (3.0 mg/kg) alone had no effect on the concentration of 5-HT and 5-HIAA 7 days following administration (Table 3).

Table 3. Effect of pretreatment with ketanserin (3.0 mg/kg) on MDMA-induced decreases in 5-HT and 5-HIAA content in the frontal cortex

		Concentration (ng/mg tissue)			
		Vehicle		MDMA	
Pretreatment	Dose (mg/kg)	5-HT	5-HIAA	5-HT	5-HIAA
Vehicle		0.27 ± 0.01	0.16 ± 0.01	0.12 ± 0.02*	0.08 ± 0.01*
Ketanserin	3.0	0.28 ± 0.01	0.17 ± 0.01	0.24 ± 0.01†	0.15 ± 0.01†

Each value is the mean ± SE of five to seven rats. Ketanserin (3.0 mg/kg, i.p.) was administered 60 min before MDMA (20 mg/kg, s.c.). The rats were killed 7 days following MDMA administration.
* Significantly ($P < 0.05$) different from vehicle–vehicle treatment group.
† Significantly ($P < 0.05$) different from vehicle–MDMA-treated group.

DISCUSSION

As previously reported by other investigators [1–3], MDMA significantly reduced brain 5-HT and 5-HIAA content 7 days following single or repeated administrations. In the present study, MDMA (20 mg/kg) significantly decreased the concentration of 5-HT and 5-HIAA in the frontal cortex 1 and 7 days following single and repeated administration. Similarly, the number (B_{max}) of 5-HT uptake sites, as identified by [^{3}H]paroxetine binding, was decreased in the frontal cortex of rats administered MDMA. These results are in agreement with previously published studies on MDMA-induced decreases in [^{3}H]paroxetine binding in the cortex [7, 20]. In contrast, neither single nor repeated injection of MDMA had an effect on the number of [^{3}H]paroxetine binding sites or 5-HT content in blood platelets 7 days following administration. These data are suggestive that blood platelets are less sensitive than serotonergic neurons in the CNS to MDMA-induced decreases in 5-HT content and [^{3}H]paroxetine binding.

The loss of 5-HT uptake sites as measured by [^{3}H]paroxetine binding has been suggested to reflect a loss of 5-HT nerve terminals [7]. Immunocytochemical studies in rodents are supportive of this hypothesis [4]. Likewise, a decrease in 5-HT and 5-HIAA concentrations in the brain following MDMA administration has been used as inferential data to support the hypothesis that MDMA destroys 5-HT nerve terminals [1, 2, 14]. However, a decrease in brain 5-HT and/or 5-HIAA content does not necessarily reflect 5-HT axon terminal damage. In the present study, MDMA significantly decreased both 5-HT and 5-HIAA content as well as the number of [^{3}H]paroxetine binding sites in the frontal cortex. Moreover, pretreatment with ketanserin significantly inhibited MDMA-induced decreases in both parameters 7 days following a single administration. These data are suggestive that the effect of MDMA on 5-HT and 5-HIAA content reflects its effect on 5-HT uptake sites. That is, the magnitude of 5-HT or 5-HIAA depletion is similar to the loss of 5-HT uptake sites as measured by [^{3}H]paroxetine binding following the administration of MDMA.

It is noteworthy that pretreatment with the 5-HT$_2$/5-HT$_{1C}$ antagonist, ketanserin, inhibited MDMA-induced decreases in brain 5-HT/5-HIAA concentrations, as well as [^{3}H]paroxetine binding. The concentration of 5-HT$_2$ binding sites in the cortex and striatum is associated with high densities of fine axon terminals arising from dorsal raphé cell bodies [21]. The administration of MDMA selectively destroys these immunocytochemically defined fine axon terminals [4]. The present finding that ketanserin pretreatment inhibited the neurotoxic effects of MDMA is suggestive that a functional association exists between 5-HT$_2$ receptors and fine axons arising from the dorsal raphé cell bodies. It is possible that a high dose of MDMA may interact directly or indirectly through the release of 5-HT with a 5-HT$_2$ or nonserotonergic ketanserin binding site [22] or both and initiate a cascade of events resulting in the destruction of these fine axon terminals. The location of this hypothesized interaction (e.g. pre- or post-synaptic) between MDMA and a 5-HT$_2$ receptor requires further study.

Previous studies in our laboratory are suggestive that ketanserin inhibits MDMA-induced activation of dopaminergic systems [14]. Dopamine has been hypothesized to play a role in the neurotoxicity of methamphetamine [23] and MDMA [24]. Preliminary data indicate that MDMA produces a significant release of dopamine in the striatum, as measured by *in vivo* dialysis, and that ketanserin blocks this effect (Nash, unpublished observation). Thus, ketanserin may block the "long-term" depletion of 5-HT by inhibiting MDMA-induced release of dopamine. If this hypothesis is correct, the 5-HT$_2$ sites at which ketanserin acts would have to increase directly or indirectly DA release.

In contrast to the marked effect of MDMA on the brain, MDMA had no effect on the number of [^{3}H]paroxetine binding sites in blood platelets 1 or 7 days following single or repeated drug administration. The only significant effect of MDMA administration was a decrease in platelet 5-HT content 24 hr following repeated injections of MDMA (Table 1). The lack of an effect of MDMA on platelet 5-HT uptake sites and content 7 days following administration could be the result of platelet turnover. That is, the life span of platelets has been estimated to be between 4 and 6 days [25–27]. Therefore, at 7 days following the last administration of MDMA the entire platelet

population could have been renewed. However, this could not account for the lack of an effect 24 hr following single or repeated administration of MDMA on the number of 5-HT uptake sites.

The basis for the difference between platelets and frontal cortex with respect to decreases in the number of [^{3}H]paroxetine binding sites and the concentration of 5-HT following the administration of MDMA is not known. The platelets and brain differ with respect to 5-HT binding proteins believed to be involved in vesicular storage [28]. MDMA may selectively interact with brain 5-HT binding proteins resulting in 5-HT depletion. Alternatively, the long-lasting depletion of 5-HT in the brain may be caused by secondary mechanisms which are not present in the periphery (e.g. dopamine release). Regardless of the mechanism(s) responsible for the difference, these data indicate that platelets are less sensitive than frontal cortex to MDMA-induced decreases in 5-HT concentration and 5-HT uptake sites. Thus, platelets appear to be a poor marker of central serotonergic changes following MDMA administration at least in rodents.

In an earlier study, Sanders-Bush and Sulser [29] found that *p*-chloroamphetamine (PCA, 10 mg/kg) does not reduce the turnover rate and biosynthesis of 5-HT in the small intestine of rats. In contrast, PCA (10 mg/kg) significantly reduces brain tryptophan hydroxylase activity in this study [23]. These authors suggest that PCA is a selective inhibitor of tryptophan hydroxylase activity in 5-HT neurons in the brain. MDMA and PCA are structurally and pharmacologically similar [30, 31]. It would appear from the present results that MDMA, like PCA, also shares the lack of an effect on peripheral measures of serotonergic function.

In summary, in accord with previous studies [1, 2, 7], a single or repeated administration of MDMA significantly decreased the concentration of 5-HT and 5-HIAA, as well as the B_{max} of [^{3}H]paroxetine binding, in the brain 7 days post-dosage. Pretreatment with ketanserin significantly attenuated MDMA-induced decreases in these parameters 7 days following a single administration. Conversely, MDMA had no effect on either 5-HT content or [^{3}H]paroxetine binding sites in the blood platelets. These data are suggestive that the mechanism(s) by which MDMA produces long-term changes in brain 5-HT content and [^{3}H]paroxetine binding sites is different from that in the platelet.

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