3,4-Methylenedioxymethamphetamine ("Ecstasy") Selectively Destroys Brain Serotonin Terminals in Rhesus Monkeys[1]

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Accepted for publication February 27, 1989

ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"), an amphetamine analog, is a "designer drug" which is being increasingly abused. The potential neurotoxic hazard of MDMA in humans was assessed by examining the effects of repeated systemic administration of MDMA on selected neurochemical and behavioral measures in rhesus monkeys. In the first study, MDMA (2.5 or 10 mg/kg twice daily for 4 days) produced selective and significant neurochemical decreases in cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and brain concentrations of serotonin and 5-HIAA. At the high dose of MDMA, a selective decrease in serotonin uptake sites (reflecting destruction of brain serotinin terminals) was observed. To determine if these changes after high dose MDMA were pharmacologic or truly neurotoxic, in a subsequent study monkeys were treated with MDMA (10 mg/kg twice daily for 4 days) and then monitored for 14 weeks. Throughout this period, CSF 5-HIAA was decreased in MDMA-treated animals but not in saline-injected controls. At the end of this period, significant decreases in the concentration of serotonin, 5-HIAA and serotonin uptake sites were observed in cerebral cortex and striatum but not in hypothalamus or spinal cord. In contrast to these widespread alterations in serotonin markers, comparable noradrenergic and dopaminergic measures in CSF and brain appeared generally unaffected. These data demonstrating potent and selective effects of MDMA on various brain serotonin parameters in rhesus monkeys suggest that the drug may produce similar effects in humans.

MDMA (Ecstasy), a ring-substituted derivative of amphetamine, represents one of a number of designer drugs which is being increasingly abused especially among college students (Peroutka, 1987). MDMA has been the subject of recent scientific and legal debate, as several psychiatrists have reported that MDMA may "enhance emotions" and "feelings of empathy" and thus serve as an adjunct in psychotherapy (Greer and Strassman, 1985). Concern about the clinical use of MDMA has arisen because the drug has a high potential for abuse (Beardsley *et al.*, 1986; Lamb and Griffiths, 1987) and has a close structural similarity to 3,4-methylenedioxyamphetamine, which selectively damages serotonin terminals in rat brain (Ricaurte *et al.*, 1985). At present, there is insufficient evidence to determine if MDMA is neurotoxic in humans.

Studies carried out in rodents have demonstrated that MDMA is toxic to brain serotonin neurons. Long-term alterations of various serotonergic parameters in brain including marked reductions in the content of serotonin and its major metabolite 5-HIAA (Battaglia *et al.*, 1987b, 1988; Schmidt *et al.*, 1986; Commins *et al.*, 1987; Stone *et al.*, 1986; O'Hearn *et al.*, 1988), decreases in tryptophan hydroxylase activity (Stone *et al.*, 1986) and destruction of serotonin terminals (Battaglia *et al.*, 1987b, 1988; O'Hearn *et al.*, 1988) have been reported after acute and repeated s.c. administration of MDMA in rats. In contrast to the marked destruction of serotonin terminals after systemic administration of MDMA, direct intracerebral injections of MDMA were without effect on cerebral cortical serotonin neurons in rats (Molliver *et al.*, 1986). These data along with the observation of marked differences in the sensitivity of MDMA-induced serotonin neurotoxicity in rats, mice and guinea pigs (Battaglia *et al.*, 1988) suggest that a peripherally produced metabolite of MDMA may be responsible for the drug's neurotoxicity. Given the major differences in the way in which rats and humans metabolize amphetamines (Caldwell, 1980), the extrapolation of data regarding the neurotoxic hazard of MDMA in rats to humans may not be valid. Several recent reports have suggested that either New World (Ricaurte *et al.*, 1988a,b,c) or Old World (Slikker *et al.*, 1988) primates may be even more sensitive than rodents to the neurotoxic effects of MDMA. In the present study, we have extended these

Received for publication November 28, 1988.

[1] This work was supported in part by funds provided by the U.S. Food and Drug Administration to the National Institute on Drug Abuse (Contract No. FDA 224-87-1301).

ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HIAA, 5-hydroxyindoleacetic acid; CSF, cerebrospinal fluid; HVA, homovanillic acid; ANOVA, analysis of variance; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol.

observations by examining the long-term effects of MDMA administration on selected neurochemical and behavioral measures in rhesus monkeys, a species in which the metabolism of amphetamines is more comparable to that seen in humans (Caldwell, 1980). We report dose-dependent neurochemical and neurodegenerative effects of MDMA in rhesus monkeys after repeated administration of the drug.

Materials and Methods

Animals and treatments. Adult male Rhesus monkeys (Macaca Mulatta) weighing 7 to 12 kg were housed under standard conditions (12-hr light/dark cycle; room temperature, 24°C) with access to food and water *ad libitum*. In the first experiment, three groups of three or four monkeys each were injected twice daily (8:00 A.M. and 4:00 P.M.) with either saline, 2.5 or 10 mg/kg of MDMA i.m. in a volume of 1 ml given in the thigh for 4 consecutive days. The low dose of MDMA (2.5 mg/kg) approximates the dose of MDMA used in humans (Greer and Strassman, 1985; Peroutka, 1987; Dowling *et al.*, 1987) and the high dose (10 mg/kg) has been demonstrated previously to produce decreases in brain concentrations of serotonin, 5-HIAA and serotonin uptake sites in rodents (Battaglia *et al.*, 1987b, 1988).

In the first experiment, CSF (2 ml) was collected by cisternal puncture with a 21-gauge needle twice during the 2 weeks before either drug or saline administration (Basal), 2 to 3 hr after the third injection (Day 2) and 16 to 18 hr after the eighth injection, *i.e.*, immediately before sacrifice (Day 5). All CSF samples were collected using sterile techniques under ketamine anesthesia. Animals were injected with ketamine (10 mg/kg i.m.), removed from the cage, sampled within 10 min and then returned to the cage. Eighteen hours after the last dose of MDMA or saline, each animal was euthanized with an i.v. injection of pentobarbital (100 mg/kg). Brains were removed rapidly and regions of interest (frontal pole, temporal pole and caudate) were dissected, frozen on dry ice and stored at −70°C until used.

In a second experiment, eight adult male Rhesus monkeys weighing 6 to 11 kg were administered either saline or MDMA (10 mg/kg) twice daily (i.m.) for 4 consecutive days and then monitored for 14 weeks before sacrifice. As in the previous experiment, CSF was collected by cisternal puncture twice during the 2 weeks before treatment for basal measures. In addition, we collected CSF 18 hr after the last treatment and every 2 to 4 weeks for 14 weeks after drug or saline administration. All animals were sacrificed at 14 weeks after treatment and brains (with brainstem and spinal cord) were removed, dissected and frozen as described above.

Assay of monoamines in CSF and brain. CSF concentrations of various monoamine metabolites were measured by high-performance liquid chromatography with electrochemical detection (Sheinin *et al.*, 1983). CSF (20 μl) was injected into a 25 cm, 5 μ, C_{18} reverse phase column with a mobile phase consisting of 0.1 M Na acetate, 0.1 mM EDTA and 6% methanol, pH 5.3.

Brain concentrations of serotonin, 5-HIAA, dopamine, HVA and norepinephrine were also measured by high-performance liquid chromotography with electrochemical detection (Mefford, 1981). For each dissected area 50 to 100 mg of tissue was sonicated in 0.5 to 1.0 ml of 0.1 N perchloric acid (4°C), then centrifuged at 12,000 × *g* for 10 min. Aliquots (20 μl) of the supernatants were injected into a 15 cm, 3 μ, C_{18} column with a mobile phase consisting of 0.2 M citric acid, 0.2 mM EDTA, 450 mg/l of octane sulfonic acid, 5 ml/l of triethylamine and 6% acetonitrile.

Assay of monoamine uptake sites. We used radioligand binding techniques with selective probes (Battaglia *et al.*, 1987b, 1988) to determine the extent of monoamine neurodegeneration by quantifying serotonin, dopamine and norepinephrine uptake sites which are highly concentrated on their corresponding nerve terminals (Kuhar and Aghajanian, 1973). Frozen brain regions were placed in 50 volumes of ice-cold assay buffer [50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl (pH 7.4 at 22°C)] and homogenized using a Brinkman polytron. The homogenate was centrifuged at 48,000 × *g* for 10 min with a subsequent resuspension and wash of the pellet. The final pellet was resuspended in the same buffer to a concentration of 15 mg wet wt./ml. [^{3}H] Paroxetine binding to serotonin uptake sites was carried out with minor modifications of the original protocol (Habert *et al.*, 1985). To obtain an estimate of the maximal density of [^{3}H]paroxetine-labeled serotonin uptake sites a saturating concentration (0.25 nM) of [^{3}H] paroxetine (K_D = 0.01 nM) was incubated with 1.5 mg of tissue in the absence or presence of 1 μM citalopram in 5 ml of the assay buffer for 2 hr at 22°C. [^{3}H]GBR 12395 binding to dopamine uptake sites was carried out as described previously (Berger *et al.*, 1985). Briefly, 0.5 nM [^{3}H]GBR 12395 was incubated with 1.5 mg of tissue in the presence or absence of 1 μM nomifensine in 1 ml of assay buffer for 1 hr at room temperature. Norepinephrine uptake sites were measured using a modification of a previously described procedure (Javitch *et al.*, 1984). [^{3}H] Mazindol (6 nM) in the presence of 30 nM GBR 12909 was incubated on ice for 60 min with 5 to 8 mg of tissue in the presence or absence of 2 μM mazindol. In all assays, the contents were filtered rapidly over 0.01% polyethyleneimine presoaked Whatman GF/C filters and washed with 15 ml of buffer. Filters were then equilibrated with 5 ml of scintillation fluid and counted in a scintillation counter. Proteins were determined according to the method of Lowry *et al.* (1951).

Behavioral scoring. In addition to these various neurochemical measures, in the first experiment the behavior of each animal was assessed using both focal observations and a portable device with a solid state memory for monitoring activity level continuously (Colburn *et al.*, 1976). Three 5-min focal observations of behavior (exploratory, passive, self-directed, stereotypy, threat and vocalization) were conducted over a 30-min period twice during the week before drug administration, 1 hr after the first (Day 1) and third (Day 2) injections, and just before sacrifice. A single observer, blind to the drug administration protocol, performed all behavioral assessments using a computer-based scoring system. Values were averaged across the three time samples to provide a daily mean for each animal.

Data analysis. CSF and behavioral data were analyzed by repeated measures ANOVA with main factors for dose and time. In the presence of significant main effects, differences from saline were determined with a post-hoc Sheffe test with confidence limits at the $P < .05$ level. Brain monoamine content and radioligand binding data were analyzed by factorial ANOVA with dose (0, 2.5 and 10 mg/kg) as the main effect in the first experiment and treatment (MDMA *vs.* saline) the main effect in the second experiment.

Results

Experiment 1: acute dose-response study with MDMA. Significant reductions in the CSF concentrations of the serotonin metabolite 5-HIAA were seen after MDMA administration [$F_{(2,6)} = 6.88$, $P = .03$] which were dependent on both the dose as well as the number of injections (fig. 1A). Whereas reductions in the CSF concentrations of 5-HIAA were evident by Day 2 of the treatment regimen (*i.e.*, after the third injection) in the 10-mg/kg treated group, significant changes in the 2.5-mg/kg treated group were observed only at Day 5 (after the eighth injection). These late decreases in CSF concentrations of 5-HIAA were associated with marked (greater than 50%), dose-dependent reductions in the concentration of serotonin (fig. 2A) in the frontal cortex [$F_{(2,6)} = 26.18$, $P < .001$] as well as in the caudate [$F_{(2,6)} = 31.51$, $P < .001$]. In these same regions, brain content of 5-HIAA was also reduced at least 50% after MDMA treatment (fig. 2B). In contrast to these effects of MDMA on brain serotonergic systems, MDMA administration was not associated with widespread or consistent changes in the levels of HVA or MHPG in CSF (fig. 1) or the concentrations of dopamine, norepinephrine or HVA in most brain areas examined (table 1). A significant dose × time interaction

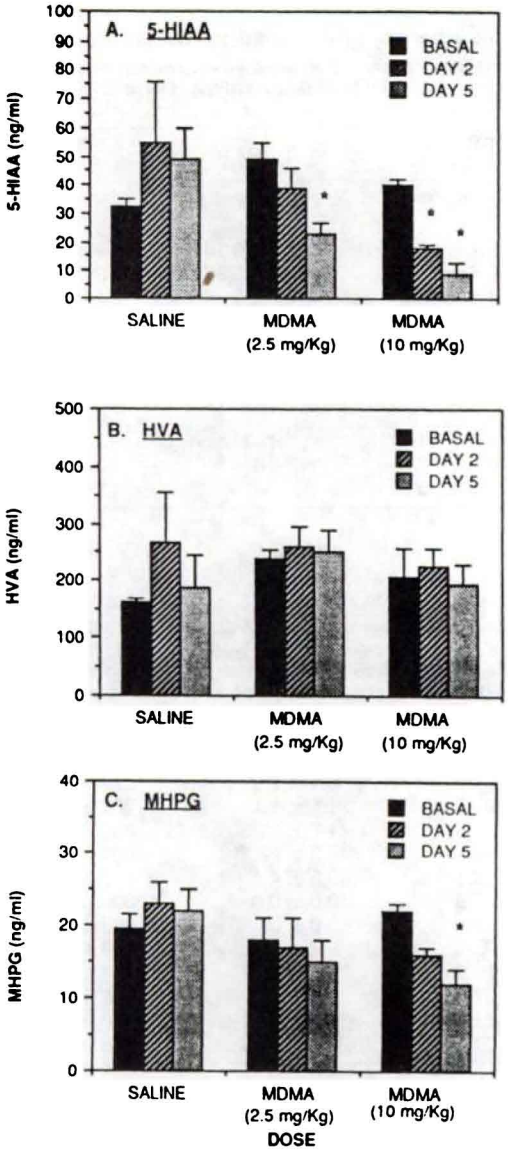

Fig. 1. Effects of MDMA treatment on CSF concentrations of (A) 5-HIAA, (B) HVA and (C) MHPG in rhesus monkeys. Data, representing the mean ± S.E.M. from three or four monkeys, were analyzed by ANOVA with repeated measures and by Scheffe post hoc test. *Significant differences at $P < .05$ from corresponding monoamine metabolite levels in saline-treated monkeys.

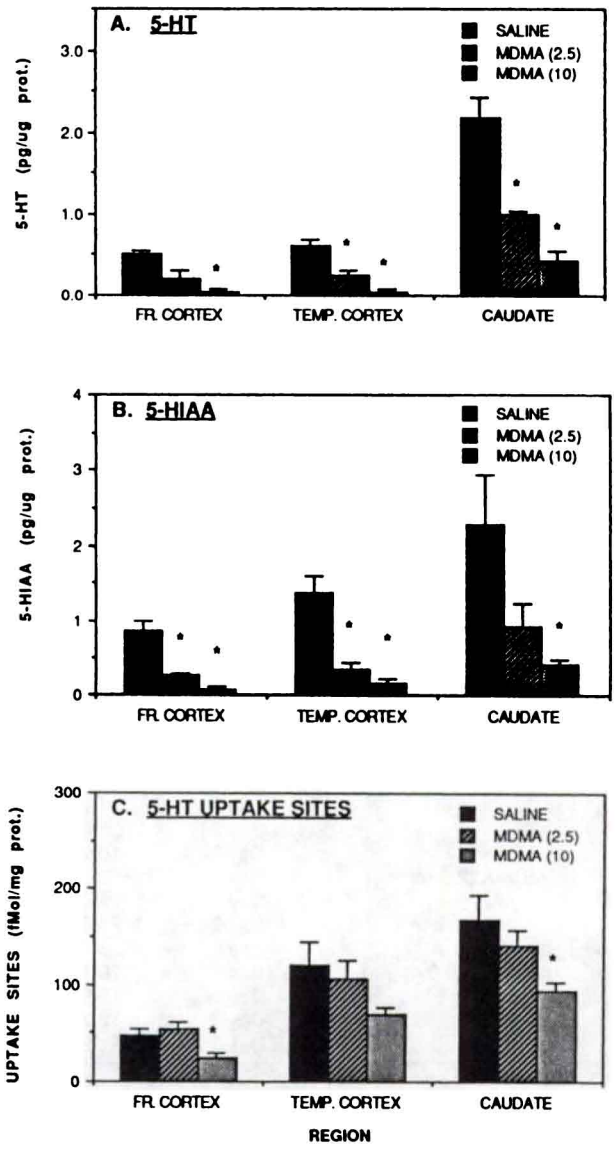

Fig. 2. Effects of repeated systemic administration of MDMA on the concentrations of (A) serotonin (5-HT), (B) 5-HIAA and (C) [^{3}H]paroxetine-labeled 5-HT uptake sites in various brain areas. Monkeys with CSF data shown in figure 1 were sacrificed at 16 to 18 hr after the last injection of MDMA or saline. Brain concentrations of 5-HT and 5-HIAA were measured by high-performance liquid chromatography with electrochemical detection; the density of 5-HT uptake sites was measured using [^{3}H] paroxetine. Data, representing the mean ± S.E.M. from three or four monkeys, were analyzed by one-way ANOVA and Scheffe post hoc test. *Significant differences at $P < .05$ from saline-injected monkeys. FR, frontal; TEMP, temporal.

[$F_{(4,12)} = 3.91$, P = .03] was observed with CSF MHPG, reflecting a decrease only after 4 days of treatment with the high dose of MDMA. Similarly, a small, statistically significant decrease in the caudate concentrations of dopamine [$F_{(2,6)} = 7.19$; P = .02] was observed after the 4-day treatment regimen of 10 mg/kg of MDMA.

These data demonstrate clearly that MDMA can significantly alter serotonergic transmission in brain; however, it is unclear whether the observed reductions in the various parameters of serotonergic function (*i.e.*, serotonin and 5-HIAA concentrations in brain and 5-HIAA concentrations in CSF) are a consequence of decreased functional activity in otherwise structurally intact neurons or are subsequent to the destruction of serotonin axons and/or terminals. Data from radioligand binding studies shown in figure 2C, demonstrate reductions in [^{3}H] paroxetine-labeled serotonin uptake sites (*i.e.*, serotonin terminals) in cerebral cortex as well as in caudate in monkeys treated with 10 mg/kg of MDMA. In contrast to the effects of MDMA on the density of serotonin uptake sites, MDMA treatment did not cause any significant reduction in the levels of [^{3}H]mazindol-labeled norepinephrine uptake sites or [^{3}H]GBR 12935-labeled dopamine uptake sites in brain (table 1).

Behavioral scores showed significant main effects for exploratory behavior [$F_{(2,6)} = 6.56$, P = .03] and passivity [$F_{(2,6)} = 5.22$, P = .05]. Exploratory behavior decreased and passivity increased after both doses of MDMA on Days 1 and 2, but were equivalent to values in saline-treated monkeys on Day 5 (table 2). Scores for stereotypy, threat, vocalization and self-directed behaviors were too low across all treatments to permit analysis;

TABLE 1

Acute effects of repeated systemic MDMA administration on dopaminergic and noradrenergic (NE) markers in rhesus monkey brain

Regional brain levels of DA, HVA and NE were measured using high-performance liquid chromatography; DA and NE uptake sites were measured using [^{3}H]GBR 12395 and [^{3}H]mazindol, respectively, as described under "Materials and Methods." Values represent the means ± S.E.M. of determinations in three to four monkeys. ND, not detectable.

Region	Dopamine	HVA	DA Uptake Sites	NE	NE Uptake Sites
	pg/μg protein	*pg/μg protein*	*fmol/mg protein*	*pg/μg protein*	*fmol/mg protein*
Frontal cortex					
Saline	0.31 ± 0.17	2.81 ± 0.27	64.1 ± 10.8	1.17 ± 0.14	40.8 ± 14.0
MDMA (2.5 mg/kg)	0.33 ± 0.22	2.83 ± 0.80	60.5 ± 12.1	1.28 ± 0.36	69.0 ± 14.0
MDMA (10 mg/kg)	0.33 ± 0.08	1.88 ± 0.23	64.4 ± 13.0	0.95 ± 0.26	47.8 ± 10.7
Temporal cortex					
Saline	0.26 ± 0.14	2.72 ± 0.65	68.5 ± 12.0	0.66 ± 0.09	141.6 ± 15.4
MDMA (2.5 mg/kg)	0.18 ± 0.06	1.55 ± 0.12	73.5 ± 7.6	0.75 ± 0.04	130.2 ± 24.2
MDMA (10 mg/kg)	0.25 ± 0.08	1.86 ± 0.24	44.2 ± 16.0	0.56 ± 0.18	201.6 ± 16.9
Caudate					
Saline	86.7 ± 3.7	66.5 ± 13.7	579.0 ± 9.0	ND	332.9 ± 75.4
MDMA (2.5 mg/kg)	77.8 ± 11.5	80.9 ± 14.1	616.0 ± 72.0	ND	442.3 ± 46.6
MDMA (10 mg/kg)	49.6 ± 3.3*	68.5 ± 7.7	571.0 ± 80.0	ND	411.6 ± 44.7

* $P < .05$ by ANOVA.

TABLE 2

Acute behavioral effects of MDMA in rhesus monkeys

Behavior was assessed by an investigator blind to the experimental protocol. Monkeys were administered either saline, MDMA (2.5 mg/kg) or MDMA (10 mg/kg) twice a day for 4 consecutive days as described in the text. Behavioral assessments were carried out in the morning twice during the week before drug or saline administration (Basal), beginning 1 hr after the first (Day 1) and third (Day 2) injections, and just before sacrifice (Day 5). Scores represent mean duration (seconds) across three five-min observations (maximum score = 300.0). Other behaviors including stereotypy, vocalization, threat and eating/drinking (operant definitions available upon request) were observed too infrequently under all conditions to permit analysis. Drug effects analyzed by repeated measures ANOVA.

Treatment	Day	Self-groom	Self-clasp	Environmental Explor.	Passive
Saline	Basal	19.7 ± 12.5	42.9 ± 5.0	6.1 ± 2.9	222.2 ± 15.5
	Day 1	37.2 ± 24.2	36.0 ± 23.6	1.1 ± 1.1	202.8 ± 31.8
	Day 2	25.0 ± 9.5	41.1 ± 31.0	21.7 ± 11.3	209.3 ± 28.9
	Day 5	16.2 ± 7.0	11.8 ± 6.0	44.2 ± 22.1	201.9 ± 38.7
MDMA (2.5 mg/kg)	Basal	89.9 ± 45.3	30.6 ± 13.1	30.5 ± 4.5	117.7 ± 38.4
	Day 1	29.9 ± 21.9	33.7 ± 23.8	0.0 ± 0.0	300.0 ± 0.0*
	Day 2	26.9 ± 8.2	5.7 ± 5.7	0.0 ± 0.0*	299.6 ± 0.4*
	Day 5	6.1 ± 3.0	27.6 ± 2.3	2.2 ± 1.3	266.1 ± 15.8
MDMA (10.0 mg/kg)	Basal	26.7 ± 10.5	20.3 ± 17.8	26.2 ± 20.7	141.1 ± 68.6
	Day 1	2.9 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	295.9 ± 2.5*
	Day 2	12.7 ± 5.1	7.7 ± 6.8	0.0 ± 0.0*	276.9 ± 11.3*
	Day 5	31.2 ± 13.4	0.0 ± 0.0	23.5 ± 11.8	220.9 ± 21.8

* $P < .05$ difference from same day saline values by post-hoc test in presence of overall drug effect.

however, several other effects were noted by the focal observer who was blind to treatment condition. From Day 1, every animal after 10 mg/kg of MDMA showed pupillary dilatation, chewing movements and myoclonic jerks. Although stationary and passive, these monkeys sat upright rather than in the typical huddled posture. By Day 2, fine motor coordination (*e.g.*, picking small bits of food off the cage bars) appeared impaired in two of the four high dose MDMA-treated monkeys. In contrast to the passivity observed on Days 1 and 2, administration of the high dose of MDMA was associated with nearly continuous activity on Days 3 and 4. This pattern can be seen on the activity monitor read-out, as shown in figure 3.

Experiment 2: long-term consequences of MDMA treatment. In the second experiment, one animal died after the sixth dose of MDMA. Necropsy on this animal revealed "bloat" or gastric distension, which was the presumed cause of death. Within the first 2 weeks, the remaining three monkeys receiving MDMA lost 12% of their pretreatment weight and failed to regain this lost weight throughout the subsequent 12 weeks (fig. 4). These changes in body weight were not observed in the saline-injected animals resulting in group differences that were statistically significant [$F_{(1,5)} = 7.33$, $P = .05$].

In the MDMA treated monkeys, CSF concentrations of 5-HIAA decreased immediately after treatment and remained decreased by approximately 40% throughout the 14-week post-treatment interval (fig. 5A). A comparison of changes from base line revealed highly significant group differences [$F_{(1,4)} = 158.3$, $P = .001$]. Group differences in HVA and MHPG concentrations in CSF were evident at base line, but there were no apparent effects of treatment in either MDMA- or saline-injected animals [$F_{(1,4)} = 1.76$, NS and 2.37, NS for HVA and MHPG, respectively) (fig, 5, B and C).

Brain concentrations of both serotonin and 5-HIAA were reduced in cortex, hippocampus and striatum, but not in hypothalamus or spinal cord (fig. 6, A and B). These reductions ranged from 50 to 90%, with confidence levels across the cortex exceeding the $P < .01$ level. Dopamine concentrations were not affected in any of the brain regions studied, but HVA was decreased significantly in motor cortex [$F_{(1,5)} = 16.2$, $P = .01$]. Norepinephrine was unchanged in cortex or cord, but actually was increased in hypothalamus [$F_{(1,5)} = 18.2$, $P = .008$] in the MDMA group (table 3).

The concentration of serotonin uptake sites was reduced by at least 50% in cortex, striatum and hippocampus but, consistent with monoamine content, was relatively unaffected in either hypothalamus or cervical cord (fig. 6C). Lumbar cord, not

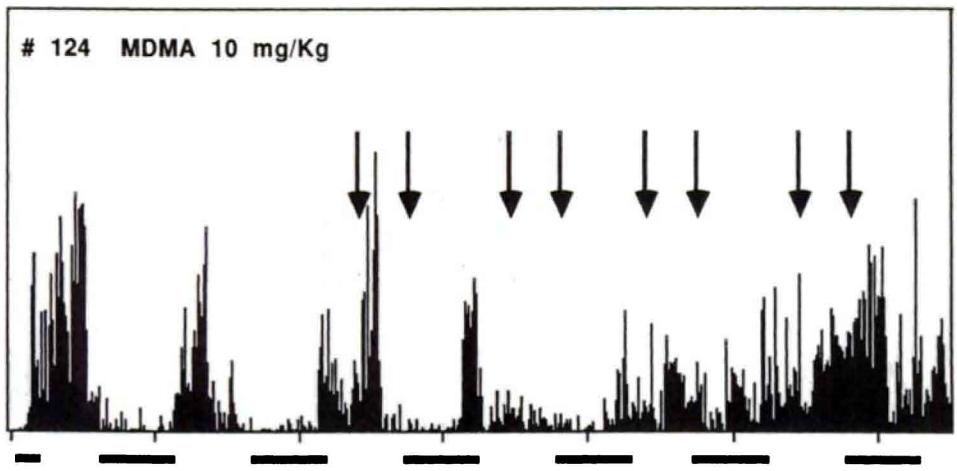

Fig. 3. Effects of MDMA treatment on continuous activity in unrestrained rhesus monkeys. Animals were fitted with nylon vests and portable monitors with a solid-state memory to record activity levels continuously for 8 days (4 days of base line and 4 days during MDMA treatment). In monkeys receiving 10 mg/kg of MDMA, activity appeared to decrease in the first 2 days, then increased on Days 3 and 4 with nearly continuous activity reflecting alteration in the normal sleep-wake cycle. Tracing, which is representative of four animals receiving 10 mg/kg of MDMA, demonstrates activity during the last 2 days of base line and 4 days of MDMA treatment in one animal. Each spike reflects total activity during a 15-min epoch (ordinate represents units of activity), arrows represent MDMA injections and horizontal bars are light-out periods.

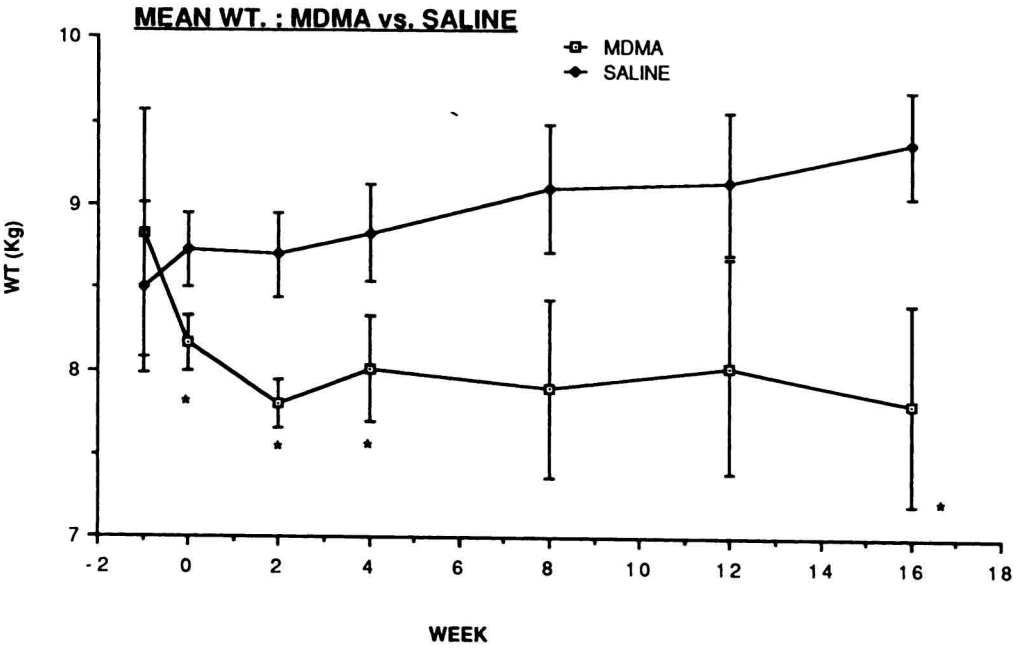

Fig. 4. Mean weights for rhesus monkeys treated with either saline or MDMA (10 mg/kg) twice daily for 4 days, then followed for 14 weeks beginning at time 0. Food and water were available *ad libitum* throughout this period. Weight change from base line was significantly different between the two groups, with * noting time points at which group differences exceed $P < .05$ for post-hoc comparisons.

shown in Figure 6C, yielded results approximating values in cervical cord. Levels of dopamine and noradrenergic uptake sites were not significantly different in any of the regions studied comparing MDMA- and saline-treated animals (table 3).

Discussion

The present study demonstrates that short-term administration of MDMA results in profound reductions in a number of brain serotonergic markers in rhesus monkeys. Specifically, MDMA administration for 4 days resulted in dose- and time-dependent decreases in 5-HIAA concentrations in CSF, and decreases in serotonin and 5-HIAA concentrations in discrete regions of brain. In addition, the marked decreases in [^{3}H] paroxetine-labeled serotonin uptake sites after 10 mg/kg of MDMA are indicative of the destruction of brain serotonin terminals. These data in rhesus monkeys parallel the observations in rodents of long-term alterations of various serotonergic parameters in brain and they are generally consistent with recently published reports of decreases in serotonin and 5-HIAA content in brain after MDMA administration to primates (Ricaurte *et al.*, 1988a,b,c; Slikker *et al.*, 1988). Our data extend these previous reports by demonstrating a selective decrease in serotonin uptake sites 4 months after treatment. Inasmuch as serotonin uptake sites were not reduced after the low dose of MDMA, the observed decreases in serotonergic parameters (*i.e.*, 5-HT and 5-HIAA) at this dose may be primarily due to suppressive effects of MDMA on functional activity in otherwise structurally intact serotonin neurons. The reduction in all serotonergic markers after administration of the high dose of MDMA (10 mg/kg) appears to be largely a consequence of destruction of serotonin terminals as these alterations persist for at least 14 weeks after treatment. The significant reductions in serotonin uptake sites in some regions (neocortex, hippocampus and striatum) but not in others (hypothalamus and spinal cord) would suggest that MDMA exhibits some degree of regional specificity in its ability to affect serotonin terminals. Further evidence for some neuroanatomical and regional specificity of the neurodegenerative effects of MDMA on brain serotonergic systems is provided by recent (Battaglia *et al.*, 1987a; O'Hearn *et al.*, 1988) data demonstrat-

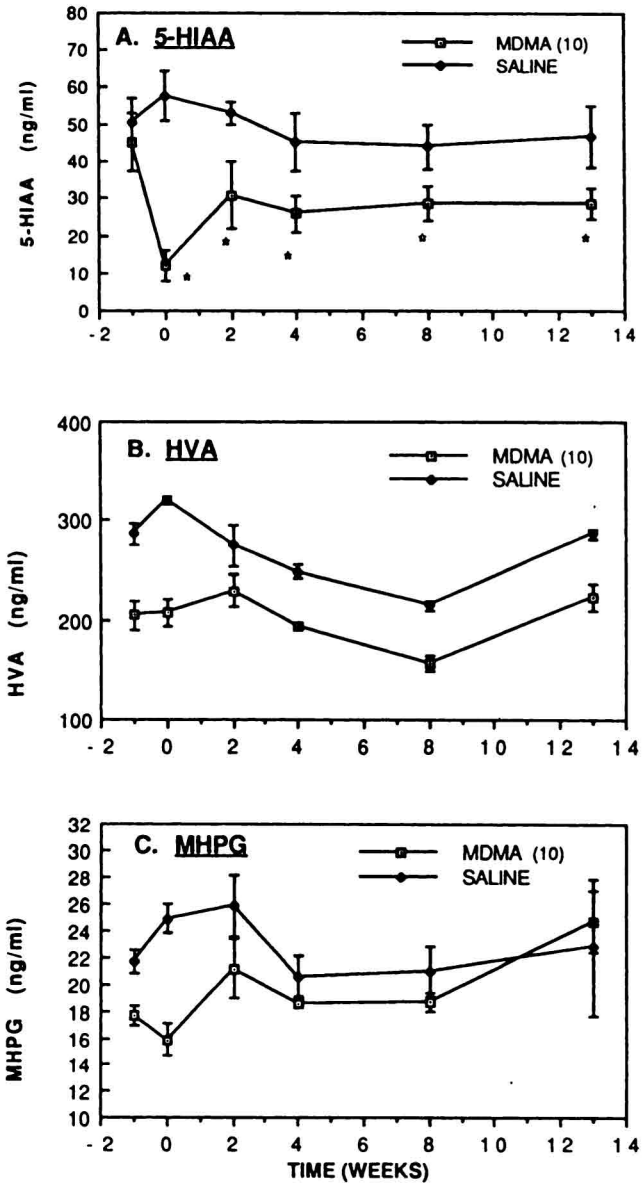

Fig. 5. Time course of CSF monoamine metabolites in rhesus monkeys treated with either saline or MDMA (10 mg/kg) twice daily for 4 days (before time 0), then followed for 14 weeks. A significant overall group difference was noted for the serotonin metabolite 5-HIAA (A), with * representing time points at which group differences exceed $P < .05$ for post-hoc comparisons. B, HVA concentrations were different at base line, but showed no significant group differences across time comparing each animal with its baseline value. Similarly, in C, MHPG concentrations were different between the two groups before treatment, but showed no significant effects of MDMA administration when individual time points were corrected for baseline values. Note that there was a trend for an initial decrease in MHPG concentration at the end of the 4-day treatment period [$F_{(1,5)} = 4.9$, $P = .07$], but this effect was not as striking as observed in the dose-response study (fig. 1C).

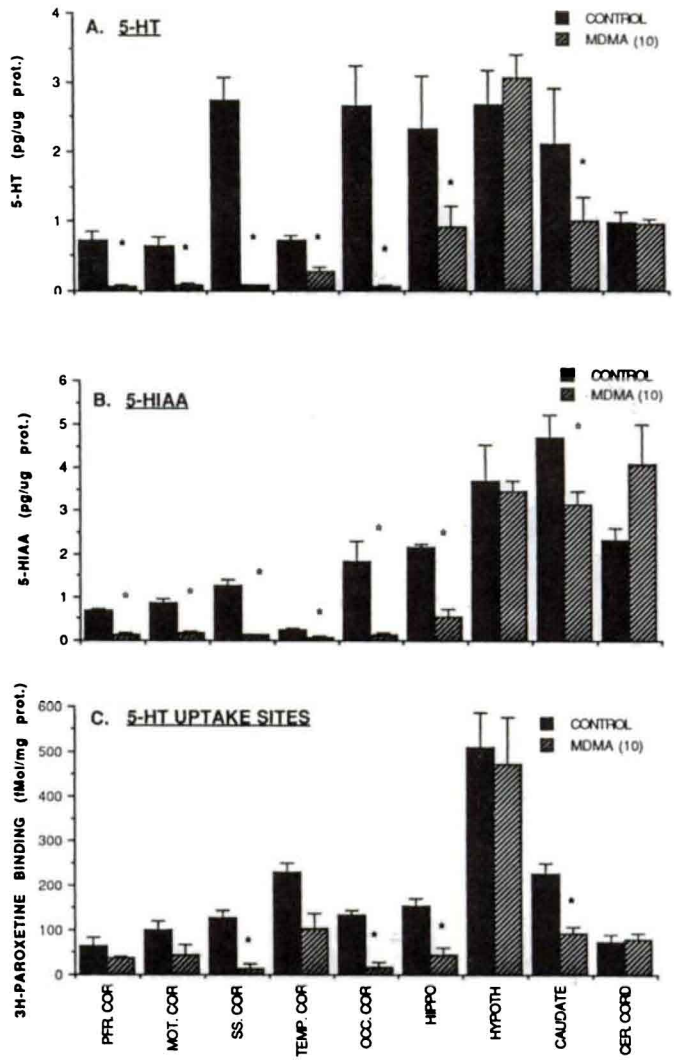

Fig. 6. Effects of repeated systemic administration of MDMA on the concentrations of (A) serotonin (5-HT), (B) 5-HIAA and (C) [^{3}H]paroxetine-labeled 5-HT uptake sites in various brain areas. Monkeys with CSF concentrations shown in figure 5 were sacrificed 14 weeks after drug or saline administration. Data represent the means of three (MDMA) or four (saline) animals with * noting significant ($P < .05$) group differences by ANOVA. pfr, prefrontal; mot, motor; ss, somatosensory; temp, temporal; occ, occipital; hipp, hippocampus; hypoth, hypothalamus (medial aspects); cer, cervical.

ing that in rodents MDMA causes preferential degeneration of serotonin terminals whereas serotonin perikarya and axons of passage are less affected.

The behavioral changes after the initial administration of MDMA (Days 1 and 2), including decreased exploratory behavior and increased passivity, were generally consistent with reports of decreased exploration and agonistic behavior that have been observed in nonhuman primates administered drugs that increase serotonin neurotransmission (tryptophan, quipazine and fluoxetine) (Raleigh *et al.*, 1980, 1986). MDMA has been reported to increase serotonin release and blocks active uptake *in vitro* (Nichols *et al.*, 1982; Johnson *et al.*, 1986), providing potential mechanisms for increased levels of synaptic serotonin that would be required to produce the behavior seen after the initial administration of the drug. In contrast to the behavior observed on Days 1 and 2, administration of the high dose of MDMA was associated with nearly continuous general activity on Days 3 and 4 (fig. 3) and disruption of the sleep-wake cycle. Although these late effects of MDMA on increased activity may appear to be a consequence of the "amphetamine-like" properties of the drug, their emergence only after the first 2 days of the high dose treatment suggest that MDMA is not behaviorally a typical amphetamine compound. Rather, these late effects of MDMA coincide with the time course of reduced serotonergic markers in brain including reductions in CSF concentrations of 5-HIAA. Drugs that reduce serotonin neu-

TABLE 3

Long-term changes in dopaminergic (DA) and noradrenergic (NE) neuronal markers after MDMA administration

Regional brain levels of DA, HVA and NE were measured using high-performance liquid chromatography; DA and NE uptake sites were measured using [^{3}H]GBR 12395 and [^{3}H]mazindol, as described under "Materials and Methods." Values represent the mean ± S.E.M. of determinations in three to four monkeys. ND, not detectable.

Region	Treatment	DA	HVA	DA Uptake	NE	NE Uptake
		pg/μg protein	pg/μg protein	fmol/mg protein	pg/μg protein	fmol/mg protein
Prefrontal cortex	Control	0.51 ± 0.09	2.60 ± 0.28	30.87 ± 44.50	1.54 ± 0.28	44.11 ± 7.27
	MDMA	0.71 ± 0.02	2.32 ± 0.41	44.42 ± 25.07	1.85 ± 0.16	37.59 ± 4.78
Motor cortex	Control	0.48 ± 0.10	3.18 ± 0.37	126.47 ± 36.60	2.64 ± 0.37	32.46 ± 10.89
	MDMA	0.42 ± 0.12	1.31 ± 0.12*	183.42 ± 99.38	3.37 ± 0.18	21.84 ± 3.35
Somatosensory cortex	Control	ND	2.47 ± 0.57	101.91 ± 37.39	2.53 ± 0.49	39.55 ± 8.13
	MDMA	ND	1.78 ± 0.13	142.55 ± 15.65	2.09 ± 0.32	30.35 ± 7.84
Temporal pole	Control	0.27 ± 0.05	2.57 ± 0.12	191.40 ± 19.04	1.13 ± 0.18	68.49 ± 12.50
	MDMA	0.37 ± 0.05	2.29 ± 0.58	136.92 ± 58.08	.98 ± 0.12	69.95 ± 14.01
Occipital pole	Control	ND	ND	41.33 ± 17.94	.53 ± 0.13	23.50 ± 7.30
	MDMA	ND	ND	27.34 ±	.52 ± 0.15	12.19 ± 5.20
Hippocampus	Control	ND	3.20 ± 0.34	124.91 ± 31.82	1.29 ± 0.32	91.45 ± 20.64
	MDMA	ND	2.31 ± 0.66	124.26 ± 14.59	1.20 ± 0.30	101.84 ± 37.53
Hypothalamus	Control	2.0 ± 0.14	26.67 ± 10.41	87.47 ± 41.40	13.42 ± 2.16	57.22 ± 10.30
	MDMA	2.51 ± 0.81	18.75 ± 5.35	113.33 ± 41.23	24.47 ± 0.52*	53.91 ± 15.82
Putamen	Control	44.70 ± 8.04	252.94 ± 19.80	709.85 ± 65.41	ND	204.55 ± 50.09
	MDMA	71.61 ± 11.14	312.86 ± 9.22	693.61 ± 51.46	ND	162.51 ± 29.93
Caudate	Control	60.27 ± 8.44	164.10 ± 12.74	667.48 ± 57.75	ND	ND
	MDMA	56.67 ± 10.76	149.23 ± 7.01	594.80 ± 6.89	ND	ND
Cervical cord	Control	ND	1.30 ± 0.01	123.27 ± 18.78	ND	30.09 ± 4.53
	MDMA	ND	1.19 ± 0.18	151.43 ± 7.16	ND	21.33 ± 4.79
Lumbar cord	Control	ND	1.52 ± 0.34	152.81 ± 13.75	ND	45.11 ± 3.23
	MDMA	ND	1.19 ± 0.19	137.28 ± 11.9	ND	35.65 ± 8.08

* $P < .05$ by ANOVA.

rotransmission (*p*-chlorophenylalanine and fenfluramine) have been associated with increased aggressive behavior, increased generalized activity and disruption of the sleep-wake cycle (Raleigh *et al.*, 1980, 1985, 1986).

In contrast to the major reductions in brain serotonin transmission after administration of MDMA, the drug did not produce any major or consistent changes in the various catecholamine markers assessed. The selectivity of MDMA's effects on brain monoamines is comparable to that seen in rodents (Battaglia *et al.*, 1987b). The decreases in the concentrations of CSF MHPG (fig. 1) and caudate dopamine (table 1) acutely after treatment with the high dose of MDMA may reflect direct effects on functional catecholamine activity rather than destruction of catecholamine nerve terminals as MDMA did not cause any significant reductions in dopamine or norepinephrine uptake sites, nor were these changes apparent in the weeks after treatment. Alternatively, inasmuch as serotonin-containing terminals are present in high concentrations in midbrain areas containing dopamine cell bodies (substantia nigra and ventral tegmental area) and in brainstem regions containing norepinephrine cell bodies (locus ceruleus) (Steinbusch, 1983; De Souza and Kuyatt, 1987), the degeneration of serotonin terminals in these regions after MDMA treatment may be indirectly responsible for these transient changes in catecholamines after the high dose of MDMA. Similarly, the increase in hypothalamic norepinephrine may reflect compensatory changes in animals with widespread serotonergic damage with concurrent weight loss.

In summary, our data demonstrate widespread destruction of serotonin terminals in rhesus monkey brain after repeated administration of 10 mg/kg of MDMA, a treatment which is associated acutely with a number of behavioral effects and some neurological deficits. Although the results did not demonstrate neurodegenerative effects on brain serotonin neurons after repeated administration of 2.5 mg/kg of MDMA, this dose is associated with significant behavioral and neurochemical effects. Although the consequences of the behavioral and neurochemical effects of MDMA for occasional recreational or "therapeutic" use of the drug in humans are unclear at present, the neurodegenerative effects may represent a risk of chronic MDMA abuse or overdose. Studies of the neurotoxicity of MDMA in rats have suggested that serotonin terminals regenerate, but the recovery occurs over a protracted period of time with significant reductions in serotonin uptake sites observed even at 6 months after a 4-day treatment regimen of MDMA comparable to that used in the present study (Battaglia *et al.*, 1988). Our results showing a persistent loss of serotonin terminals in rhesus monkeys comparable to previous reports in rodents suggest that similar long-term effects might be observed in humans.

Acknowledgments

The authors thank Drs. Michael J. Kuhar and Joseph F. Contrera for helpful discussions regarding the design of the study, Mr. Ron Harbaugh for technical assistance and Ms. Terrie Pierce and Ms. Katherine Compton for manuscript preparation.

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