

Plasma Levels of Parent Compound and Metabolites after Doses of Either *d*-Fenfluramine or *d*-3,4-Methylenedioxymethamphetamine (MDMA) that Produce Long-Term Serotonergic Alterations

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Abstract

Plasma levels of parent compounds and metabolites were determined in adult rhesus monkeys after doses of either 5 mg/kg *d*-fenfluramine (FEN) or 10 mg/kg *d*-3, 4-methylenedioxymethamphetamine (MDMA) i.m. twice daily for four consecutive days. These treatment regimens have been previously shown to produce long-term serotonin (5-HT) depletions. Peak plasma levels of $2.0 \pm 0.4 \mu\text{M}$ FEN were reached within 40 min after the first dose of FEN, and then declined rapidly, while peak plasma levels ($0.4 \pm 0.1 \mu\text{M}$) of the metabolite norfenfluramine (NFEN) were not reached until 6 h after dosing. After the seventh (next to last) dose of FEN, peak plasma levels of FEN were 35% greater than after the first dose while peak NFEN-levels were 500% greater. The $t_{1/2}$ for FEN was 2.6 ± 0.3 h after the first dose and 3.2 ± 0.2 h after the seventh. The estimated $t_{1/2}$ for NFEN was more than 37.6 ± 20.5 h. Peak plasma levels of $9.5 \pm 2.5 \mu\text{M}$ MDMA were reached within 20 min after the first dose of MDMA, and then declined rapidly, while peak plasma levels ($0.9 \pm 0.2 \mu\text{M}$) of the metabolite 3,4-methylenedioxymethamphetamine (MDA) were not reached until 3–6 h after dosing. After the seventh (next to last) dose of MDMA, peak plasma levels of MDMA were 30% greater than the first dose while peak MDA levels were elevated over 200%. The $t_{1/2}$ for MDMA was 2.8 ± 0.4 h after the first and 3.9 ± 1.1 h after the seventh dose. The estimated $t_{1/2}$ for MDA was about 8.3 ± 1.0 h. Variability in plasma levels of MDMA and MDA between subjects was much greater than that for FEN and NFEN. This variability in MDMA and MDA exposure levels may have lead to variability in the subsequent disruption of some behaviors seen in these same subjects. There were 80% reductions in the plasma membrane-associated 5-HT transporters 6 months after either the FEN or MDMA dosing regimen indicating that both treatments produced long-term serotonergic effects.

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INTRODUCTION

d-Fenfluramine (FEN) is an anorectic previously prescribed to treat obesity until the FDA recalled it over concerns about possible peripheral toxicity

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(Doogan, 1982; Roche et al., 1992; Cacoub et al., 1995). In addition to the possible peripheral toxicity of FEN seen in humans, it can evoke a prolonged depletion of serotonin (5-HT) in brain regions of rats and monkeys at higher doses (Harvey and McMaster, 1975; Clineschmidt et al., 1976, 1978; Kleven et al., 1988; Appel et al., 1989; Molliver and Molliver, 1990; Ricaurte et al., 1991; McCann et al., 1994). Furthermore, FEN-induced depletion of 5-HT is potentiated by hyperthermia (Clausing et al., 1998; Stewart et al., 1997; Malberg and Seiden, 1997), and neuronal degeneration can occur if the hyperthermia is substantial (Schmued et al., 1999).

Plasma levels of FEN and its metabolite norfenfluramine (NFEN) have been reported for the rat after neurotoxic doses of FEN (Clausing et al., 1998). However, the present study is the first to determine plasma levels of FEN and NFEN in monkeys given neurotoxic doses. Knowledge of the plasma levels and resultant effects of an agent could be used as a basis for dose response modeling and species extrapolation. This approach has been used in the risk assessment procedures for several neurotoxicants (Slikker et al., 1996; Gaylor and Slikker, 1990).

Like FEN, *d*-3, 4-methylenedioxymethamphetamine (MDMA) and *d*-3, 3,4-methylenedioxyamphetamine (MDA) at high doses can produce long-term depletions of 5-HT in the brains of laboratory animals (Ali et al., 1993; Frith et al., 1987; Gibb et al., 1986; Insel et al., 1989; Ricaurte et al., 1985; Slikker et al., 1988; Steele et al., 1994). Furthermore, these 5-HT depletions are highly influenced by the degree of hyperthermia that occurs in rats during MDMA exposure (Broening et al., 1995; Farfel and Seiden, 1995). In addition, temperature-dependent dopamine depletions are produced by MDMA in monkeys and rodents (Miller and O'Callaghan, 1994; Ricaurte et al., 2002). Although MDMA is not normally prescribed for clinical use, it has been used experimentally in humans for both sanctioned and unsanctioned psychotherapy (Greer and Tolbert, 1998; Peroutka et al., 1988; Liester et al., 1992). Therefore, it is important to know the plasma levels of MDMA and its active metabolite MDA associated with its long-term alterations in serotonergic systems. The plasma levels of MDMA and MDA in rats given neurotoxic doses of MDMA have been previously determined (Chu et al., 1996). However, the present study is the first to determine these levels in monkeys given "neurotoxic" (long-term depletions in the neurotransmitter levels and plasma membrane-associated transporters for 5-HT) doses of MDMA.

Plasma levels of the parent compound and metabolites were determined in adult rhesus monkeys admin-

istered neurotoxic doses of either 5 mg/kg *d*-fenfluramine (FEN) or 10 mg/kg *d*-methylenedioxy-methamphetamine (MDMA) i.m. twice daily for four consecutive days. The long-term depletions in 5-HT plasma membrane-associated transporter binding to paroxetine are presented to show that depletions in 5-HT transporters are even more pronounced than 5-HT depletions in brain tissues. Specific behavioral effects and the long-term brain 5-HT depletions produced by this regimen have been previously reported (Frederick et al., 1998). Additional evaluations of the behavioral data were performed here to see if differences between subject plasma levels of drug or metabolite might have been associated with differences in subsequent behavioral performance.

METHODS

Subjects

Nine adult male rhesus monkeys (*Macaca mulatta*) between 8 and 19 years of age and weighing from 7 to 10 kg served as subjects. Six animals had previously been trained to perform behavioral tasks in the NCTR operant test battery (OTB) and had done so for several years; the three control subjects had not. During the year preceding this study, one subject in the FEN group (M79) and two in the MDMA group (M81 and M436) had been exposed to cocaine and ditolyguanidine during separate studies to determine the acute behavioral effects of these agents. All of these animals, however, had been drug free for at least 3 weeks prior to the current study. Two subjects in the FEN group (M72 and M73) and one in the MDMA group (M71) were drug naïve with the exception of routine clinical exposures to ketamine for tuberculosis testing and health checks. The control animals served as controls for the neurochemical analyses and also for determining blank plasma backgrounds for drug and metabolite levels.

Drug Administration and Temperature Monitoring

d-Fenfluramine hydrochloride (purchased from Research Biochemicals International, Natick, MA) and MDMA [supplied by the National Institute on Drug Abuse (NIDA)] were dissolved in saline and administered i.m. Either 5 mg/kg FEN or 10 mg/kg MDMA was given twice daily at approximately 8:00 a.m. and 4:00 p.m. for four consecutive days. It was previously reported that the animals used in this study

did not become hyperthermic during either MDMA or FEN exposure (Frederick et al., 1998) but neither the methods used nor the temperatures were published. To determine the core body (rectal) temperatures in these studies, a 2 mm flexible thermistor probe (YSI Inc., Yellow Springs, OH) was inserted 10–12 cm into the rectum and securely taping the distal portion of the probe to the monkeys' tail, and the temperatures were monitored using a model 5830R Thermistor thermometer (Digitec, Marion, OH). Temperatures were determined for 8 h after the first injection on all four dosing days for either FEN or MDMA administration.

Tissue Collection and Analysis for 5-HT Uptake Sites

Animals were sacrificed for brain tissue collection as described by Frederick et al. (1998). Dissection of the prefrontal cortex used for determination of 5-HT uptake sites was performed as described by Brown et al. (1979) using the atlas of Szebenyi (1970). The dissected tissue from the prefrontal cortex was immediately frozen on dry ice and then transferred to storage at -70°C . The frozen tissue was later rapidly thawed, and membranes were prepared for determination of 5-HT uptake sites using the [^3H]paroxetine binding methods as adapted from Marcusson et al. (1988). Protein content of the membranes used for the binding experiment was determined by the method of Lowry et al. (1951).

Plasma Extraction and HPLC-Quantitation of FEN, MDMA and Metabolites

Blood samples were obtained by venipuncture using heparinized syringes, transferred to glass centrifuge tubes and centrifuged at $1000 \times g$ for 10 min to obtain plasma. Plasma extraction and quantitation of FEN, NFEN, MDMA and MDA levels was accomplished using the methods of Clausing et al. (1997) with the amended procedures given in the following description. Ten microliters of a $25 \mu\text{M}$ fluoxetine in water and 0.1 M HCl solution was added to $90 \mu\text{l}$ of plasma as an internal standard. The plasma was then made basic with $100 \mu\text{l}$ 0.1 M borate buffer (pH 10.6) and extracted with ethyl acetate as described by Clausing et al. (1997). However, to improve the derivatization procedure, the nitrogen dried analyte (the ethyl acetate plasma extract) was reconstituted with $15 \mu\text{l}$ of 0.25 M H_3PO_4 and vortexed multiple times over 1 h to ensure the re-suspension of the analytes prior to derivatization with dansyl chloride. Also, the procedure for the removal of excess dansyl chloride reaction

by-products using a strong anion exchange resin was modified by increasing to 15 min the time that the resin was mixed/shaken with the reaction products. The analyte was then allowed to sit at 5°C for 1–3 days before HPLC analysis, which reduced background reaction by-product peaks that can interfere with analysis. The dansyl chloride derivatives were stable for over 1 month if kept at 5°C .

A Supelcosil LC-18 ($4.6 \text{ mm} \times 15 \text{ cm}$) column, running a step gradient with 50% KH_2PO_4 (0.05 M , pH 5.5) + 50% acetonitrile (mobile phase A) versus 25% KH_2PO_4 (0.05 M , pH 5.5) + 75% acetonitrile (mobile phase B) was used for HPLC isolation. The HPLC elution gradient is shown as follows.

Time (min)	Gradient slope	Buffer A (%)	Buffer B (%)
0–5	Isocratic	100	0
5–7	Step	60	40
7–12	Linear	45	55
12–14.5	Linear	35	65
15	Step	0	100
22	Step	100	0
30	End run	100	0

The separated eluents were detected via fluorescence at 470 nm after excitation at 345 nm. The limit of detection for all compounds was less than 5 pmol but greater than 2.5 pmol for the $90 \mu\text{l}$ of plasma assayed. The retention times were 10.26 min (MDA), 13.36 min (MDMA), 14.57 min (NFEN), 19.53 min (FEN) and 22.08 min (fluoxetine). Fluoxetine hydrochloride from Ely Lilly (Indianapolis, IN, USA) was used as an internal standard. Because this method does not distinguish between *l*- and *d*-enantiomers we refer to MDA, MDMA, FEN and NFEN-levels in this paper, rather than *d*-FEN and *d*-NFEN-levels, although it is reasonable to assume that these enantiomers do not appreciably racemize after administration of *d*-FEN.

A pharmacokinetic modeling analysis was performed on the plasma levels of FEN and MDMA using the WinNonlin[®] (Scientific Consulting Inc., Apex, NC) pharmacokinetic software package. The results indicated that a single compartmental analysis was an appropriate model for the plasma levels of both FEN and MDMA (see section “Results” for specifics). The graphical, model independent, method of Ritschel (1986) was also utilized to calculate the absorption and elimination half-life values, area under the plasma concentration–time curve (area under the curve (AUC)_{0–8}), and apparent volume of distribution

(aVd) from the plasma levels of FEN and MDMA. The $t_{1/2}$ yielded by this model was compared to that of WinNonlin[®]. The elimination rate constant (beta) was obtained by linear regression of the terminal data points from the ln plasma concentration–time plot. The half-life was obtained by calculation ($-0.693/\text{rate constant}$). The $\text{AUC}_{0 \rightarrow 480}$ was calculated based on the trapezoid rule; $\text{AUC}_{0 \rightarrow 8}$ was obtained from $\text{AUC}_{0 \rightarrow 480} + (\text{concentration at 480 min}/\text{beta})$. The aVd was calculated from $\text{dose}/(\text{beta} * \text{AUC}_{0 \rightarrow 8})$ assuming that 100% of the i.m. dose was available for distribution. The time (t_{max}) of the maximum concentration (C_{max}) was obtained by observation.

In order to estimate the formation and elimination of the metabolite (MDMA $\rightarrow$ MDA and FEN $\rightarrow$ NFEN), plasma data were simulated using the physiologically based pharmacokinetic (PBPK) model of Young et al. (2001). This system simulated the eight i.m. doses

(input) and the sampling of the plasma for parent compound and metabolite after the first and eighth doses. The metabolite elimination rate constant was obtained from the linear portion of the extended simulation curves. The formation rate constant was obtained by subtracting the elimination phase from the original data and regressing on these constructed data points. Much of the pharmacokinetic data generated by these methods can be seen in Figs. 2 and 4.

Operant Behavior

Monkeys that received the neurotoxic dosing regimen of FEN and MDMA were part of an operant behavioral study in which one of the tasks they performed was an incremental repeated acquisition task designed to model learning. The details of this task are described elsewhere (Paule, 1990, 2001; Schulze et al.,

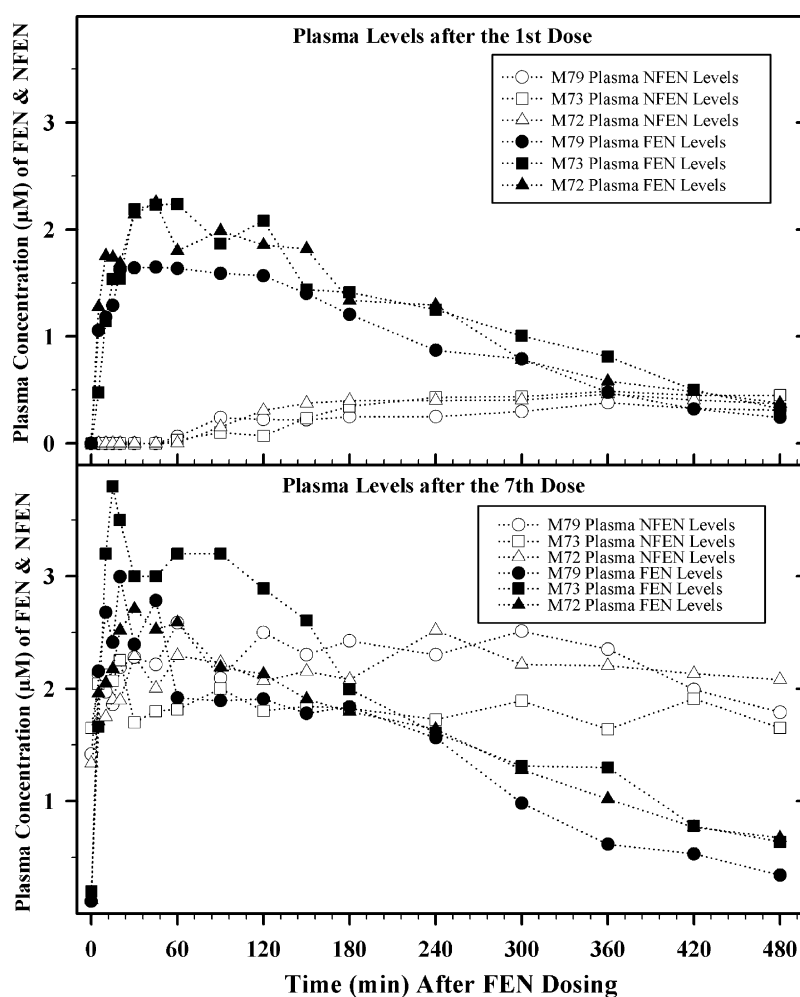


Fig. 1. Individual plasma levels of FEN and NFEN after exposure to the first and next to last dose during a “neurotoxic” exposure to FEN. Plasma levels of FEN and NFEN for each monkey were determined at 0, 5, 10, 15, 20, 30, 45 min and at 1 h intervals up to 8 h after the first (top graph) or the seventh dose (bottom graph) of 5 mg/kg i.m. FEN. Monkey identification numbers are given in the legend (e.g. M79).

1988). Briefly, monkeys performed the learning task every other day as part of a battery of operant tasks administered daily Monday through Friday of each week. Monkeys were required to learn simple lever sequences followed by progressively more difficult sequences within a given session, and a different set of sequences was presented each test day. A measure of the overall level of performance of this task is represented by a percent task completed (PTC) endpoint that describes the extent to which the monkeys were able to “learn” progressively more difficult sequences in a given session.

RESULTS

The plasma levels for FEN and NFEN after the first and seventh (last) doses of 5 mg/kg (equivalent to 18.8 $\mu\text{mol/kg}$) FEN for each of the three monkeys given FEN are shown in Fig. 1. The average plasma levels for FEN and NFEN after the first and seventh (last) doses are shown in Fig. 2 (without the SEM bars to facilitate viewing) along with simulated levels of both compounds. Peak FEN plasma levels of $2.04 \pm 0.35 \mu\text{M}$ were reached within 40 min after the first dose, and then declined rapidly while peak

plasma levels of $0.43 \pm 0.08 \mu\text{M}$ of NFEN, the metabolite of FEN, were not reached until 6 h after dosing. After the seventh dose the peak plasma levels of FEN were 35% greater at $2.74 \pm 0.75 \mu\text{M}$ than the peak after the first dose. The area under the curve for FEN increased from $10.12 \pm 1.50 \mu\text{M h}$ after the first to $15.42 \pm 2.85 \mu\text{M h}$ for the seventh dose. Peak NFEN-levels were elevated over five-fold after multiple dosing to $2.23 \pm 0.39 \mu\text{M}$. The calculated $t_{1/2}$ for FEN was $2.57 \pm 0.31 \text{ h}$ after the first dose. This did not change significantly after eight doses ($t_{1/2} = 3.22 \pm 0.24 \text{ h}$). The apparent volume of distribution for FEN was $6.85 \pm 0.22 \text{ l/kg}$ as determined from data for the first dose. Although the precise $t_{1/2}$ for NFEN could not be directly calculated from the data due to the nearly constant levels that appear in the 8 h monitoring period shown in Fig. 1, it was estimated to be $37.6 \pm 20.5 \text{ h}$ from the PBPK model simulation and subsequent graphical analysis.

The individual plasma levels for MDMA and MDA after the first and seventh dose of 10 mg/kg (equivalent to 43.5 $\mu\text{mol/kg}$) MDMA for all 3 subjects are shown in Fig. 3. The mean plasma levels for MDMA and MDA after the first and seventh dose of 10 mg/kg MDMA (equivalent to 43.5 $\mu\text{mol/kg}$) are shown along with simulated levels of both compounds in Fig. 4.

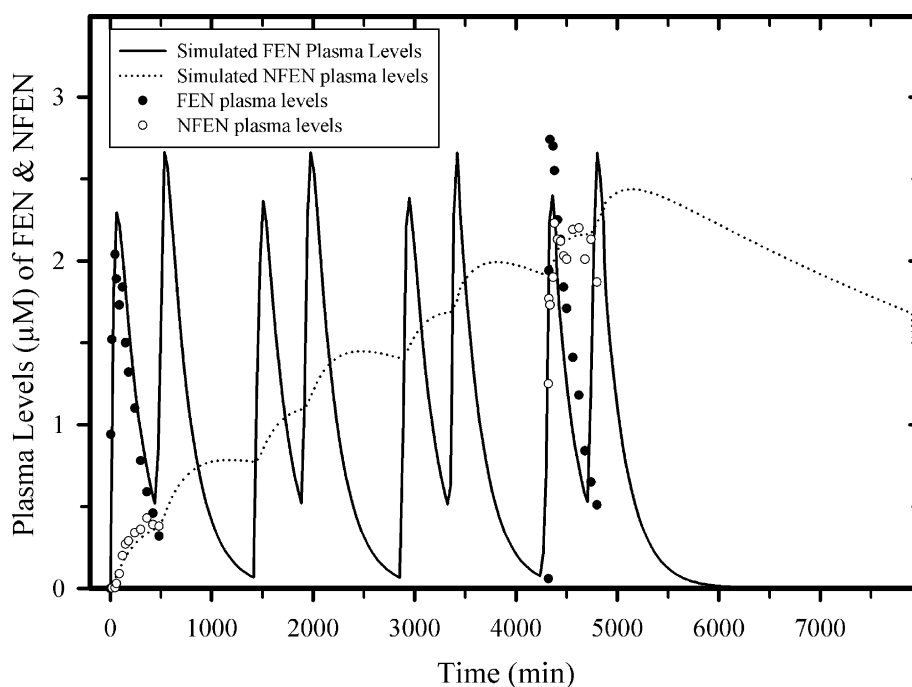


Fig. 2. The group real and simulated plasma levels of FEN and NFEN observed during a “neurotoxic” exposure to FEN. The mean analytically determined plasma levels of FEN (filled circles) and NFEN (open circles) after the first and seventh dose are shown for the mean data for the three monkeys given 5 mg/kg FEN. The S.E.M. was omitted for clarity of presentation. The solid line represents the simulated levels of FEN and NFEN over the entire dosing period that was generated from the analytically determined levels (see section “Methods”).

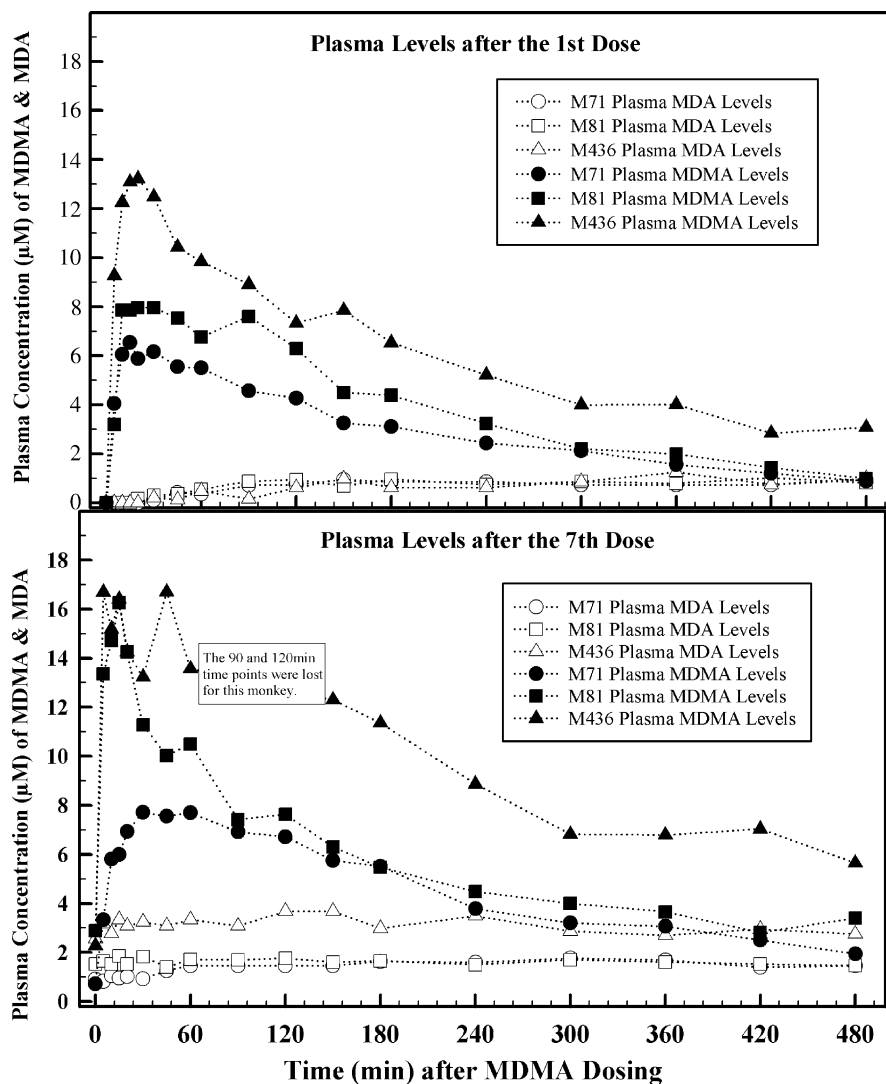


Fig. 3. Individual plasma levels of MDMA and MDA after exposure to the first and next to last dose during a “neurotoxic” exposure to MDMA. Plasma levels of MDMA and MDA for each monkey were determined at 0, 5, 10, 15, 20, 30, 45 min and at 1 h intervals up to 8 h after the first (top graph) or the seventh dose (bottom graph) of 10 mg/kg i.m. *d*-MDMA. Monkey identification numbers are given in the legend (e.g. M71).

Peak plasma levels of $9.50 \pm 2.53 \mu\text{M}$ MDMA were reached within 20–40 min after the first dose and then declined rapidly. The peak plasma levels of the 3,4-methylenedioxymphetamine metabolite of $0.92 \pm 0.23 \mu\text{M}$ were not reached until 2–3 h after dosing. Data for the seventh dose of MDMA show peak plasma levels of MDMA that are 30% greater ($12.88 \pm 4.87 \mu\text{M}$) than after the first dose, and the AUC increased from 40.96 ± 18.83 to $75.45 \pm 39.06 \mu\text{M h}$. $2.30 \pm 0.98 \mu\text{M}$. The calculated $t_{1/2}$ for MDMA was 2.84 ± 0.37 h after the first dose and this increased after eight doses to 3.94 ± 1.13 h). The estimated apparent volume of distribution for MDMA was 4.81 ± 1.68 l/kg as determined from data for the first dose. Although the precise $t_{1/2}$ for MDA could not be

directly calculated during the 8 h monitoring period follow dosing (Fig. 3), it was indirectly determined from the PBPK model simulation and subsequent graphical analysis to be 8.3 ± 1.0 h. Unlike the data for subjects receiving FEN, a wide variability in plasma levels for both MDMA and MDA was seen (Fig. 3). The plasma levels of MDMA and MDA for subject M436 were almost twice those for M71 while the plasma levels of both compounds for subject M81 were between those for M436 and M71. These differences were nearly the same for plasma samples collected after both the first and seventh doses.

Multiple doses of either 5 mg/kg FEN or 10 mg/kg MDMA produced 80% reductions in the plasma membrane-associated 5-HT transporter (Fig. 5) 6 months

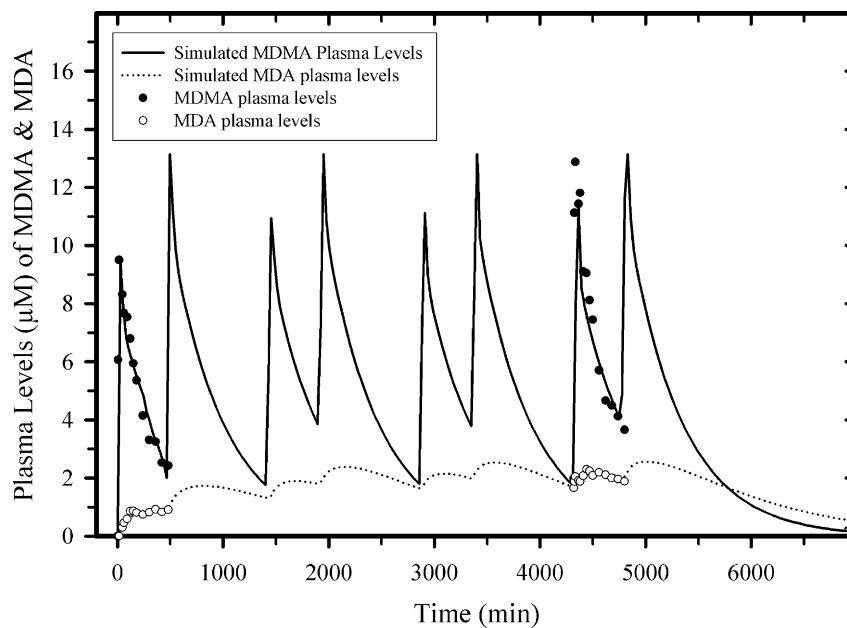


Fig. 4. The group real and simulated plasma levels of MDMA and MDA observed during a “neurotoxic” exposure to MDMA. The mean analytically determined plasma levels of MDMA (filled circles) and MDA (open circles) after the first and seventh dose are shown for the mean data for the three monkeys given 10 mg/kg MDMA. The S.E.M. was omitted for clarity of presentation. The solid and dotted lines, respectively, represent the simulated levels of MDMA and MDA over the entire dosing period that was generated from the analytically determined levels (see section “Methods”).

after drug exposure indicating that both doses were supra-threshold for neurotoxic effects. There was little variability in these long-term decreases in transporter density among the subjects of either group despite the fact that there was a wide variability in the plasma levels of parent compound and metabolite in the group receiving MDMA.

The mean body temperatures dropped between 1.5 and 2 °C over the 8 h after the first dose of either FEN or MDMA administration on all four dosing days. Only the body temperatures for 1st and 4th day of dosing are shown in Fig. 6 for clarity sake. However, the temperature profiles for FEN and MDMA were the same on the 2nd and 3rd day as the other 2 days. Although the baseline (0 min time point) is about 1.5 °C higher than

the dosing and blood collecting procedures that were on going during temperature monitoring.

In light of the significant differences between the plasma levels of MDMA and MDA noted in the subjects receiving MDMA, the changes in behavioral data reported earlier for these animals (Frederick et al., 1998) after neurotoxic insult was re-evaluated. Here, the average percent of the specific learning task that was completed each session was determined for the three behavioral sessions conducted during the week prior to MDMA dosing and compared to that for the three behavioral sessions conducted the week immediately following MDMA dosing. Finally, rectal temperatures of the monkeys remain within normal limits throughout the 1 week dosing period in all the animals (Frederick et al., 1998).

Monkey ID	Peak [MDMA] and MDA first dose (µM/µM)	Peak [MDMA] and MDA seventh dose (µM/µM)	Pre-MDMA PTC	Post-MDMA PTC	% Decrease
M436	13.2/1.3	16.7/3.7	77.2	11.7	84
M81	8.0/1.0	14.7/1.7	42.8	26.7	37
M71	6.6/0.9	7.71/1.8	30.0	20.3	32

expected for humans, this is well within the expected control range for monkeys (Lane et al., 1996). It is not clear whether the noted drop in body temperature is related to the effects of FEN and MDMA or represents the normal physiological response of these animals to

Comparison of these behavioral data with the plasma levels of MDMA during dosing indicates that the monkeys with higher plasma levels of MDMA had larger decreases in the percent task completed for the learning task. Within a month, this behavioral disruption was no

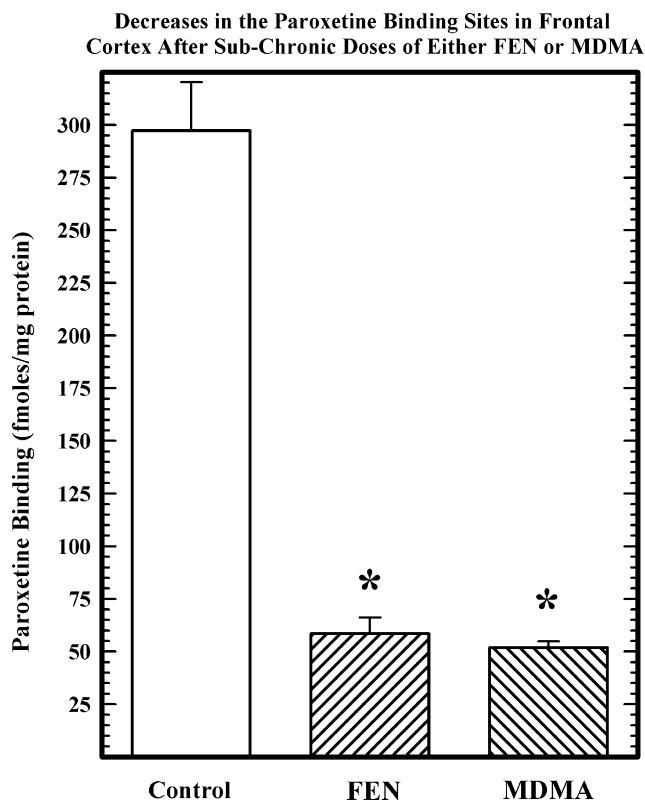


Fig. 5. Group levels of 5-HT plasma membrane-associated transporter levels in frontal cortex after exposure to a “neurotoxic treatment” regimen of either FEN or MDMA. 5-HT transporters in plasma membranes from the frontal cortex were determined using [3 H]paroxetine binding to the P2 pellet obtained from this region more than 6 months after exposure to either FEN or MDMA. Transporter levels are expressed as the mean $\pm$ S.E.M. Star means significantly differs from control at the $P < 0.001$ level.

longer evident under non-drug conditions. However, as a group, the animals were less sensitive to disruption of behavioral performance by a subsequent non-5-HT depleting challenge dose of 1.75 mg/kg MDMA.

DISCUSSION

With respect to the plasma levels of FEN and NFEN seen in our studies after multiple twice daily treatments with FEN, it is apparent that our data are commensurate with what would be predicted from previous studies that also evaluated plasma levels in rhesus monkeys (Caccia et al., 1995). A single oral dose of 2 mg/kg FEN resulted in peak plasma levels of 0.061 μ M (14 ng/ml) FEN and 0.451 μ M (97 ng/ml) NFEN in those studies. The peak NFEN-levels reported by Caccia et al. (1995) are about the same as those reported in the present studies. Although the

peak FEN levels in the Caccia study are 20-fold less, the much lower FEN levels are likely due to the different routes of administration (oral administration in the Caccia studies and intramuscular in the current study). The $t_{1/2}$ of 2.3 h for FEN reported by Caccia et al. (1995) is similar to our value of 2.6 h. However, the $t_{1/2}$ of 10.7 h that they report for NFEN is significantly less than the over 37.6 h estimated in the present study. The difference in NFEN $t_{1/2}$ we observed versus that observed by Caccia et al. may be due in part to either the differences in the methods used to determine $t_{1/2}$ (s) (we used a less precise indirect method) or the fact that the values of the present study were determined after multiple doses. Nonetheless, the $t_{1/2}$ must have been more than 20 h in our present studies just to account for the more than five-fold increase in blood levels we observed by the seventh dose.

Caccia et al. (1995) estimated plasma levels of 0.12–0.15 μ M NFEN in rhesus monkeys after chronic dosing with 0.5 mg/kg FEN which is one-tenth the dose used in the present study. These predicted plasma levels are about one-tenth of the levels (1.25–2.20 μ M NFEN) observed in the current study after 4 days of dosing. The plasma levels of FEN ranged significantly higher in the present study (less than 0.05–1 μ M) compared to the 0.015 μ M estimated by Caccia et al. (1995) but again this difference is primarily due to differences in the route of administration. The bioavailability of FEN is about 60% in humans after oral administration (Cheymol et al., 1995), which, if also true in monkey, would explain the higher combined levels of FEN and NFEN observed after i.m. administration compared to the combined FEN and NFEN-levels seen after oral dosing.

The peak plasma levels of 2.7 μ M FEN and 2.2 μ M NFEN seen in monkeys in our studies are about 20 times the steady state levels predicted for humans given 15 mg/kg orally twice daily (estimates based on data from earlier monkey studies, Caccia et al., 1995). However, these predictions were made using plasma levels of FEN and NFEN that were only about half of the 20 ng/ml (0.075 μ M) FEN and 8 ng/ml (0.035 μ M) NFEN plasma levels determined after an oral dose of 15 mg in humans by Cheymol et al. (1995). Because the half-lives for FEN and NFEN are almost 1 day in humans, levels in humans after chronic dosing are about four-fold those seen after a single dose. Therefore, the therapeutic levels of FEN and NFEN may only be about one-tenth those necessary in monkeys to produce long-term serotonergic changes. Because of the rapid first pass metabolism of FEN to NFEN seen after oral administration in monkeys

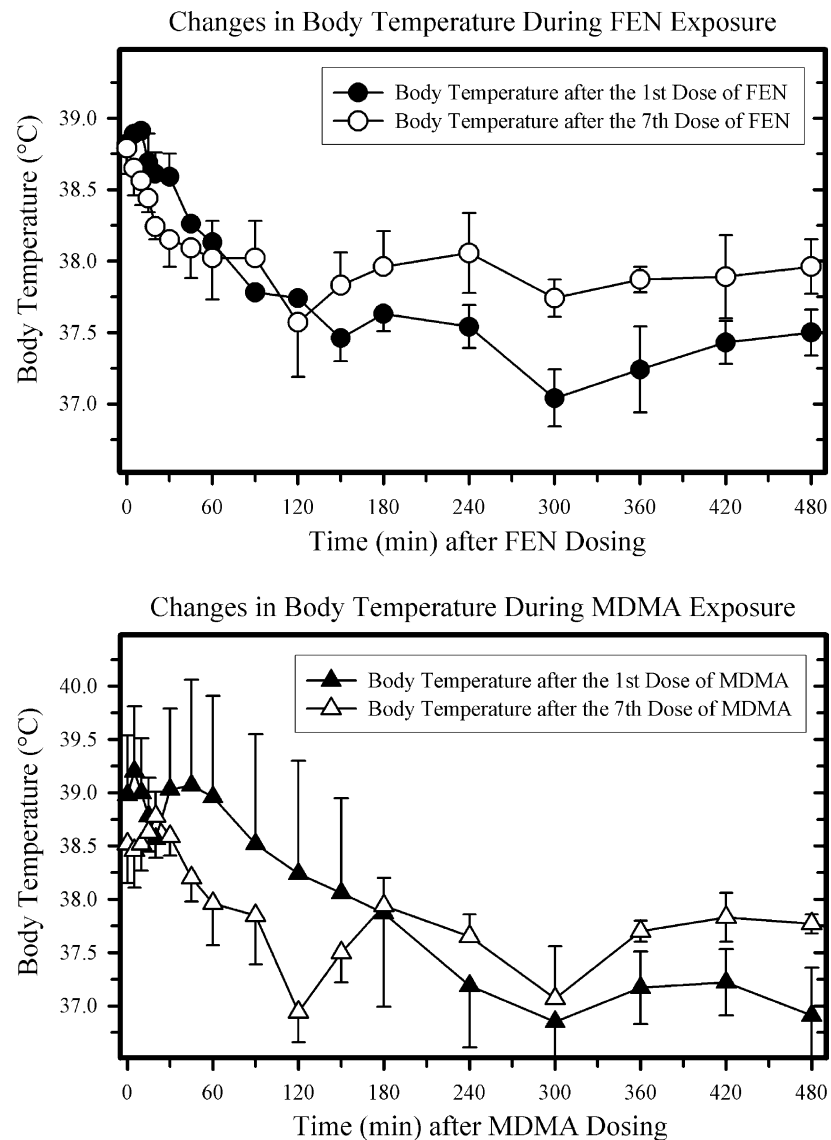


Fig. 6. The body temperature profiles observed after either FEN or MDMA administration. The average body temperatures $\pm$ S.E.M., after either FEN (top graph) or MDMA (bottom graph) are shown for the same time points (5 min–8 h) for which blood samples were collected. Temperatures obtained after the first dose are shown with either filled circles (FEN) or filled triangles (MDMA) while data obtained after the seventh dose on the 4th day are shown with open circles (FEN) or open triangles (MDMA).

(Caccia et al., 1995), the i.m. route in this species more effectively models the pharmacokinetics seen in humans after oral dosing. However, even with i.m. administration of FEN and very short dosing intervals, the human plasma FEN and NFEN-levels obtained after oral dosing cannot be precisely mimicked in monkeys (Caccia et al., 1995; Campbell, 1995).

The plasma levels of FEN and NFEN attained in our studies were sufficient to produce pronounced long-term depletions in 5-HT levels (Frederick et al., 1998) and plasma membrane transporters that lasted more than 6 months after exposure. This long-term effect on serotonergic and dopaminergic levels and plasma membrane transporters in the brain has been previously well docu-

mented in both laboratory animals and humans and termed neurotoxicity by several investigators (Appel et al., 1989; McCann et al., 1994, 1998; Molliver and Molliver, 1990; Ricaurte et al., 1991, 2002). Others have argued that, at doses producing plasma levels approximately equal to those seen with therapeutic doses, the effects of FEN on serotonergic systems are readily reversible, are not truly neurotoxicity, and/or are not very likely to occur in humans (Kalia, 1990; Baumgarten et al., 1992; Campbell, 1995).

Nonetheless, overt neurotoxicity in the form of neurodegeneration is seen in rats after high doses of FEN when significant hyperthermia occurs (Schmued et al., 1999). In addition, hyperthermia has been reported to

greatly exacerbate MDMA-induced damage to dopaminergic terminals in monkeys (Ricaurte et al., 2002). Although somatic neurodegeneration has not yet been reported in primates, studies more closely monitoring temperature and following a more rigorous time course of histological evaluation may show this to occur. The plasma levels of FEN and NFEN we observed in monkeys after a single dose of 5 mg/kg are similar to the plasma levels of 2.6 μ M FEN and 0.6 μ M NFEN seen in rats after a single dose of 10 mg/kg i.p. FEN which is sufficient to produce neurodegeneration in this species when hyperthermia occurs (Clausing et al., 1998; Schmued et al., 1999). Thus, if severe hyperthermia were to occur in monkeys after dosing with 5 mg/kg FEN, the potential for neurodegeneration exists. However, in the present studies hyperthermia did not occur during FEN exposure (Frederick et al., 1998). Furthermore, dopamine depletions were not observed (data not shown) which, from the work of Ricaurte et al. (2002), would have been expected if the monkeys had become hyperthermic. Therefore, somatic neuronal degeneration would not necessarily be predicted, and the changes in frontal cortex 5-HT and plasma transporter levels we observed were not dependent on hyperthermia.

Using the dosing paradigm of the present studies, the peak plasma levels were between 9 and 13 μ M for MDMA and greater than 2 μ M for MDA. The dosing regimen used (10 mg/kg, i.m. twice daily for 4 days) was sufficient to reduce 5-HT plasma membrane transporters by 80% after more than 6 months. In rats, doses of between 20 and 40 mg/kg MDMA (Chu et al., 1996) were reported to produce similar levels of MDMA. These doses are 2–4 times those necessary to produce long-term serotonergic and plasma membrane-associated transporter depletions in the rat when hyperthermia occurs (Broening et al., 1995). These results indicate that plasma levels of MDMA and MDA that are 50% lower than those seen in the present studies may be sufficient to produce neurotoxicity in the presence of hyperthermia.

The plasma levels of MDA attained in the monkey after the 10 mg/kg doses of MDMA are more than 10-fold below the 35–145 μ M that were seen in fatal human poisonings from MDA during the early 1970s (Cimbura, 1972). However, the plasma levels of 9–13 μ M MDMA seen in the current study are near the range of the 15–30 μ M levels (*d*- and *l*-enantiomers combined) reported to be fatal when hyperthermia occurs (Brown and Osterloh, 1987; Walubo and Seger, 1999; Moore et al., 1996). It should be noted that in these human studies the MDA levels were five times

(above 10 μ M) those seen in the monkeys of the present study. One report indicates that much lower plasma levels of MDMA can result in hyperthermia and fatality under certain environmental conditions (Henry et al., 1992). However, it is also possible that other substituted amphetamines such as paramethoxyamphetamine may have been contaminating the illicit “ecstasy” (Byard et al., 1998) and responsible for the induction of fatal hyperthermia. Furthermore, demethylenated MDMA and 6-hydroxy-MDMA-like metabolites of MDMA which were not determined in the present studies, can be formed and maybe potentially more neurotoxic and lethal (Chu et al., 1996; Elayan et al., 1992).

The 40–125 mg doses which have been used in either psychotherapy or as “safe” doses for illicit recreational use, have been reported to produce plasma levels of 1 μ M or less (Mas et al., 1999; Fallon et al., 1999). These levels are about one-tenth the levels seen in monkeys showing long-term decreases in 5-HT levels and plasma membrane-associated transporters. However, the monkey plasma MDMA and MDA levels may be considerably above the threshold levels necessary to produce long-term depletions. Doses of 10 and 20 mg/kg MDMA in the rat have been shown to produce long-term 5-HT depletions when hyperthermia occurs but these doses result in plasma levels of 5 μ M or less (Broening et al., 1995; Chu et al., 1996). Thus, levels considerably below 10 μ M might be expected to produce long-term depletions in monkeys, and is more plausible given the fact that hyperthermia greatly exacerbates dopaminergic depletions and terminal damage in monkeys (Ricaurte et al., 2002) and mice (Miller and O’Callaghan, 1994). In humans, where adverse interactions with hyperthermia, other drug and individual differences in metabolism of MDMA are possible, predicting a “safe” dose of MDMA for the general population becomes problematic. Particularly in light of the fact that plasma membrane 5-HT transporters are significantly altered by illicit MDMA use (McCann et al., 1998). From previous MDMA studies involving monkeys (Ali et al., 1993) and from our present studies, it is very likely that the plasma level of MDMA necessary to produce long-term depletions of 5-HT levels and transporters would be less than 10 μ M.

The 10 mg/kg dose of MDMA may have been supra-threshold for maximal long-term depletions in 5-HT tissue and transporter levels but not for temporary disruption of behavioral performance. Collectively, the data from above tables suggest that plasma levels of MDMA during neurotoxic dosing may be predictive of subsequent short-term performance deficits in

certain operant behavioral tasks. However, within a month these disruptions were no longer present. The behavioral responses were altered when subjects were subsequently challenged with MDMA, and, in this case, the monkeys are less sensitive to the disruptive effects of MDMA than they were prior to treatment with the neurotoxic regimen. Therefore, reduced 5-HT levels and plasma membrane transporters do not affect baseline or 'unchallenged' performance of behavioral tasks designed to model learning and other brain functions after a short recovery period.

In summary, doses of 5 mg/kg FEN and 10 mg/kg MDMA produced plasma levels of parent compounds and metabolites in rhesus monkeys that are approximately 10 times those seen in humans after administration of therapeutic doses (FEN) or "safe" illicit doses of (MDMA). Although the plasma levels of MDMA we observed in the monkey approached those potentially lethal for humans, it would be expected that some humans abusing MDMA would attain and survive such exposures. The 5 mg/kg dose of FEN produced long-term depletions in 5-HT tissue and plasma membrane-associated transporter levels in the monkeys in the absence of hyperthermia. However, in light of data previously reported for rats, it would be predicted that the plasma levels of FEN produced by 5 mg/kg FEN have the potential to produce frank neurodegeneration in the monkey if significant concomitant hyperthermia were to occur. Although short-term disruption of learning behavioral tasks was observed with MDMA, persistent behavioral deficits were not noted when the monkeys were in a drug free state.

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