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Cardiovascular and Neuroendocrine Effects and Pharmacokinetics of 3,4-Methylenedioxymethamphetamine in Humans[1]

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ABSTRACT

The cardiovascular and neuroendocrine effects and pharmacokinetics of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") were assessed in a double-blind, randomized, crossover, and controlled (placebo and amphetamine) clinical trial. Eight men with experience in the recreational use of MDMA participated in four 10-h experimental sessions with a 1-week washout period. Single oral doses of 125 mg and 75 mg of MDMA, 40 mg of amphetamine, and placebo were given. Both MDMA doses significantly increased blood pressure (increases of 40 mm Hg in systolic blood pressure), heart rate (increases of 30 beats/min), and pupillary diameter (mydriasis) as compared with placebo. Oral temperature did not show significant changes in any drug-active condition. Plasma cortisol levels showed a statistically significant increase after MDMA administration. Prolactin levels only increased after high dose of MDMA. C_{max} values for 125-mg and 75-mg MDMA doses were 236.4 and 130.9 ng/ml, and T_{max} was observed at 2.4 and 1.8 h, respectively. Elimination half-life was 8.6 h and 7.7 h for high and low MDMA doses, respectively. Amphetamine half-life was 15 h. Between 8 and 9% of the doses of MDMA appeared in plasma in the form of 3,4-methylenedioxyamphetamine. The important cardiovascular effects observed after MDMA administration in laboratory conditions at rest (increases of 40 mm Hg in systolic blood pressure and 30 beats/min in pulse rate) could be relevant in terms of toxicity in real-life conditions (e.g., crowded places and physical activity).

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a synthetic amphetamine derivative with potent effects on serotonergic neurotransmission. The drug, synthesized in 1912 but never marketed, became popular in the 1970s and 1980s due to the induction of feelings of euphoria, friendliness, closeness to others, and empathy after its use. These properties have been named "entactogen" by some authors (Camí and Farré, 1996) and seem the basis of its use in psychotherapy during the 1980s until it was included in the schedule I psychotropic substances list (Steele et al., 1994).

MDMA has become increasingly popular in Europe and North America over the past 10 years. It is primarily consumed by young people in large dance and music environments ("raves") and sometimes in small social settings (Camí and Farré, 1996). Undesirable effects associated with the recreational use of MDMA include loss of appetite, jaw clenching, trismus, bruxism, headache, nausea, sweating, muscle aches, fatigue, and insomnia. MDMA can also cause acute toxic reactions with tachycardia, hypertension, arrhythmia, panic attack, and psychosis. A number of case reports of severe intoxication and death after MDMA abuse have been reported (Dowling et al., 1987; Henry et al., 1992). Acute medical complications include malignant hyperthermia, seizures, cerebral hemorrhage, hepatitis, rhabdomyolisis, disseminated intravascular coagulation, and acute renal failure (McCann et al., 1996). The use of MDMA together with other serotonergic compounds may trigger the appearance of a serotonin syndrome, which is characterized by confusion, fever, shivering, diaphoresis, ataxia, hyperreflexia, myoclonus, and diarrhea (Demirkiran et al., 1996). Studies in rats and primates have shown neurotoxicity after the administration of single and repeated doses of MDMA secondary to long-lasting depletion of serotonin (5-HT) and damage of 5-HT axon terminals with loss of 5-HT uptake sites. Although reinnervation may occur after several months, it is abnormal in some brain areas. Decreased 5-hy-

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ABBREVIATIONS: AMPH, amphetamine; CV, coefficient of variation; GH, growth hormone; 5-HT, serotonin; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; SBP, systolic blood pressure; DBP, diastolic blood pressure; AUC, area under the concentration-time curve.

droxyindole acetic acid concentrations in the cerebrospinal fluid from monkeys (Ricaurte et al., 1988) and regular users (McCann et al., 1994) and a reduction of 5-HT transporter have been described in both animals and humans (McCann et al., 1998). Because doses of MDMA taken by recreational users are close to those causing neurotoxicity in monkeys, there is a great concern regarding its possible neurotoxic effects in humans (Green and Goodwin, 1996).

Little is known about the pharmacology of MDMA in humans. Most data have been obtained from noncontrolled studies, epidemiological surveys, and intoxication cases. Only two studies of MDMA administration in a placebo-controlled clinical trial have been reported. In the study of Grob et al. (1996), very low doses were given orally (0.25–1.0 mg/kg as a single dose) as compared with those used for recreational purposes (75–100 mg), whereas in the study of Vollenweider et al. (1998), a dose of 1.7 mg/kg (119 mg for 70 kg of weight) was administered to 13 MDMA-naïve healthy volunteers. In both studies, MDMA caused an increase in heart rate and blood pressure and induced positive psychological states. Data on pharmacokinetics and drug disposition are also limited. The main metabolites are 3,4-methylenedioxyamphetamine (MDA) obtained by *N*-demethylation of the parent compound and 4-hydroxy-3-methoxymethamphetamine obtained by demethylenation followed by *O*-methylation (Helmlin et al., 1996). In a study in which a single dose of 50 mg of MDMA was administered to a 40-year-old male volunteer, 2 to 10% of the dose was recovered in the urine as MDA (Verebey et al., 1988). In another report, plasma and urine concentrations of MDMA were described after the administration of 1.5 mg/kg to two subjects participating in psychotherapy sessions (Helmlin et al., 1996). In a recent letter by Henry et al. (1998), a graphic display of MDMA kinetics after the administration of 40 mg to eight subjects is shown. Some authors have suggested a possible role of MDMA metabolites in neurotoxic effects (Green and Goodwin, 1996).

To determine cardiovascular and neuroendocrine effects and pharmacokinetics of MDMA in healthy volunteers, we conducted a double-blind, randomized, crossover trial using amphetamine and placebo as control medications. MDMA was administered orally as a single dose. Doses were selected in the range of those reported by recreational users.

Experimental Procedures

Subjects. Male subjects were recruited by "word of mouth". Eligibility criteria required the recreational use of MDMA on at least five occasions. Eligible persons were initially interviewed by one of us (M.F.) to exclude concomitant medical conditions and psychiatric disorders. Subjects who fulfilled the inclusion criteria were then interviewed by a psychiatrist (structured clinical interview for DSM-IV) to exclude individuals with history of major psychiatric disorder (including schizophrenia, psychosis, and major affective disorder). Each participant underwent a general physical examination, routine laboratory tests, urinalysis, and 12-lead ECG.

A total of 14 volunteers were included, 6 in the pilot phase and 8 in the final study. The eight participants in the final study had a mean age of 26.5 years (range 21–30), mean weight of 74.4 kg (range 66–83), and mean height of 178 cm (range 169–186). All but two subjects were current smokers. Their average consumption of alcohol was 2 units/day (1 unit = 8 g of ethanol), and all of them had previous experience with cannabis, cocaine, and methamphetamine consumption. None had a history of abuse or drug dependence according to DSM-IV criteria (except for nicotine dependence) or any medical or psychiatric adverse reaction after MDMA consumption.

All volunteers gave their written informed consent before inclusion in the study and were paid for their participation. The study was conducted in accordance with the Declaration of Helsinki, approved by the Ethical Committee of our institution, and authorized by the "Dirección General de Farmacia y Productos Sanitarios" (number 95/297) of the Spanish Ministry of Health.

Study Design. To avoid the subjective effects of expectancy, subjects were informed they would receive single oral doses of stimulants (one of which could be MDMA), sedatives, or placebo. They participated as outpatients in four 10-h experimental sessions. The washout period was 1 week for all sessions; only one session was scheduled with a 2-week washout period. Before starting the first experimental session, a training period of about 4 to 5 h was carried out to familiarize volunteers with the testing procedures. The study design was double-blind, randomized, crossover, and controlled. Treatment conditions were randomly assigned using a balanced 4 × 4 Latin-square design. In the four study sessions, subjects arrived at the laboratory at 8 AM after an overnight fast and had an indwelling i.v. catheter inserted into an s.c. vein in the forearm of the nondominant arm. Thereafter, they remained seated in a quiet room throughout the session. Drugs were administered around 9.30 AM. At 3 and 6 h after drug administration, subjects had a light meal. Tobacco smoking was permitted 4 h after drug administration. The room temperature during the trial remained constant between 20 and 21°C. Urine drug screens were conducted before each experimental session and were all negative for opioids, cocaine, and amphetamines.

Drugs. The four drug conditions were as follows: 125 mg of DL-MDMA, (MDMA 125), 75 mg of DL-MDMA (MDMA 75), 40 mg of DL-amphetamine (AMPH 40) (Centramine; Miquel SA, Barcelona, Spain), and placebo. MDMA was supplied by the Spanish Ministry of Health. Drugs were prepared by the pharmacy service of our hospital as identically appearing opaque, white soft-gelatine capsules and administered in fasting state with 200 ml of tap water (two capsules each time). The doses of MDMA and amphetamine were selected according to a pilot study phase carried out in three different pairs of subjects ($n = 6$) where different doses of MDMA (50, 100, and 150 mg), amphetamine (20, 30, 35, and 40 mg) and placebo were administered (Camí et al., 1998). The low MDMA dose produced mild effects, and the high dose induced marked effects in terms of intensity of the subjective effects and increases in blood pressure and pulse rate. The 150-mg dose was therefore considered too risky, and the 125-mg and 75-mg doses were selected for the trial.

Study Methods. Subjects were phenotyped for CYP2D6 activity using dextromethorphan. The dextromethorphan-dextrorphan metabolic ratio was used to classify subjects as extensive or poor metabolizers (Schmid et al., 1985). All participants were extensive metabolizers.

Noninvasive heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and oral temperature were recorded at 30 min and immediately before drug administration (time 0) and at 15, 30, 45, 60, and 90 min and at 2, 3, 4, 6, 8, 10, and 24 h after drug administration using a Dinamap 8100-T vital signs monitor (Critikon, Tampa, FL). Pupillary diameter was recorded with a Haab pupil gauge (Pickworth et al., 1998) at 0 and at 30, 60 and 90 min, and 2, 3, 4, 6, 8, 10, and 24 h after drug administration. For safety reasons, ECG was continuously monitored during all the session using a Dinamap Plus vital signs monitor (Critikon, Tampa, FL). Subjective effects were also measured (data not shown).

An indwelling i.v. catheter was inserted in a peripheral vein, and 0.9% sodium chloride solution was infused at a rate of 20 ml/h. Blood was collected in each session to preserve the double-blind masking of the study. Blood samples (6 ml, heparinized tubes) were obtained for analysis of MDMA, MDA, and amphetamine at 0 and at 15, 30, 45, 60, and 90 min and 2, 3, 4, 6, 8, 10, and 24 h after drug administra-

tion. In addition, a 4-ml sample for hormone analysis (cortisol, prolactin, growth hormone) was drawn and collected in nonheparinized tubes at 0 and at 30, 60, and 90 min as well as 2, 3, 4, and 6 h after drug administration. Samples were centrifuged at 3000 rpm for 10 min and at 4°C. Plasma and serum was removed and frozen at −20°C until analysis.

Assays. For MDMA and MDA analysis, plasma aliquots of 1 ml were allowed to thaw at room temperature and were processed, together with a calibration curve, after addition of 100 ng of 3,4-methylenedioxypropylamphetamine as internal standard. Extraction was carried out using Bond Elut Certify columns (Varian, Harbor City, CA). The pH of the samples was adjusted by adding 1 ml of 0.1 M phosphate buffer (pH 6) and passed through the columns, which had been previously conditioned by passing sequentially 2 ml of methanol and 0.1 M phosphate buffer. Columns were washed consecutively with 1 ml of acetic acid and 6 ml of methanol. MDMA and MDA were eluted with 2 ml of chloroform with 2% of ammonium hydroxide. Eluates were evaporated to dryness at 30°C under nitrogen stream, and the residue was redissolved in 100 μl of methanol. One microliter was injected onto the chromatographic system. Analyses were performed in a gas chromatograph (HP-5890 series II; Hewlett-Packard, Palo Alto, CA) equipped with a nitrogen-phosphorous detector. Separation was carried out using a cross-linked 5% phenylmethylsilicone capillary column, 12 m × 0.2 mm i.d., and 0.33-μm film thickness (Ultra 2; Hewlett-Packard). Helium was used as carrier gas at a flow rate of 0.8 ml/min. Air and hydrogen detector flows were set at 100 and 4 ml/min, respectively. Injector and detector temperature was set at 280°C. The initial oven temperature was maintained at 70°C during 2 min and programmed to 100°C at 30°C per min, then to 200°C at 20°C per min, and finally to 280°C at 25°C per min. Samples were injected in splitless mode. Calibration curves were linear over a concentration range of 20 to 400 ng/ml for MDMA and of 5 to 100 ng/ml for MDA. The quantification limits were 5.2 ng/ml and 2.6 ng/ml for MDMA and MDA, respectively. Recoveries obtained were 85% for MDMA and 91% for MDA. Interday precision values, expressed as coefficient of variation for specific added concentrations, were lower than 8.1% and 9.2% for MDMA and MDA, respectively. Interday accuracy values, expressed as percentage error of concentration found as compared with target added concentrations, were lower than 2.3% for MDMA and 4.5% for MDA. Intraday precision values were lower than 6.9% and 9.9% for MDMA and MDA, respectively. Intraday accuracy values were lower than 1.1% for MDMA and 5.8% for MDA.

For amphetamine analysis, plasma aliquots of 1 ml were allowed to thaw at room temperature and were processed, together with a calibration curve, after addition of 25 ng of a deuterated analog (d_8-amphetamine) as internal standard. Samples were treated adding 0.4 N sodium hydroxide and 0.5 ml of a saturated solution of sodium chloride and then extracted with 5 ml of terc-butylmethylether by rocking mixing for 20 min. The organic phase was separated and treated with 20 μl of *N*-methyl-bis-trifluoroacetamide to prevent amphetamine losses during the evaporation process. After evaporating to dryness under a nitrogen stream at 30°C, samples were derivatized with 50 μl of *N*-methyl-bis-trifluoroacetamide at 70°C during 15 min, and 2 μl were injected onto the chromatographic system. Gas chromatography-mass spectrometry analysis was performed in a Hewlett-Packard 5890A gas chromatograph coupled to a model 5970 quadrapole mass spectrometer (Palo Alto, CA). The samples were injected in splitless mode into a 25 m × 0.2-mm i.d., 0.33-μm film thickness methylsilicone column (Ultra 1; Hewlett-Packard). The injector operating in splitless mode and the interface were operated at temperatures of 280°C. The oven temperature was initially programmed at 80°C for 2 min and increased at 20°C until 280°C. Helium was used as carrier gas at a flow rate of 0.7 ml/min. The mass spectrometer was operated by electron impact ionization and in the selected ion-monitoring acquisition mode. Ions *m/z* 118, *m/z* 140 for amphetamine and *m/z* 126, *m/z* 143 for d_8-amphetamine were selected for quantification. Calibration curve was linear over the 2.5 to 70 ng/ml concentration range. Limit of quantification was 1.4 ng/ml, and 92% of amphetamine was recovered after the extraction process. Interday precision (expressed as coefficient of variation for specific added target concentrations) and accuracy (expressed as percentage error of concentration found as compared with target-added concentrations), were lower than 6.3% and 6.2%. Intraday precision and accuracy were lower than 7.5% and 6.2%, respectively.

Plasma cortisol concentrations were determined by fluorescence polarization immunoassay (Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. A good correlation has previously been shown between fluorescence polarization immunoassay results and reference methods (Kobayashi et al., 1979). The intra-assay coefficients of variation (CV) were 2.92 and 2.60% for low (4.00 μg/dl) and high (40.00 μg/dl) controls. Interassay CV were in the range of 17.43% and 4.74% (1.81) for low and high controls, respectively. The assay sensitivity is reported to be 0.45 μg/dl.

Prolactin plasma concentrations were determined by a microparticle enzyme immunoassay (Abbott Laboratories, Chicago, IL) using an IMx^R instrument and following the manufacturer's instructions. A good correlation has previously been shown between microparticle enzyme immunoassay results and reference methods (Bodner et al., 1991). The microparticle enzyme immunoassay assay sensitivity is reported to be 0.6 ng/ml. Intra-assay CV were 1.61% and 1.02% for low (8.00 ng/ml) and high (40.00 ng/ml) controls, and interassay CV 3.56% and 5.39% (2.05) for low and high controls, respectively. The IMx^R prolactin calibrators have been assigned values relative to the World Health Organization 2nd International Standard (83/562) where 1 ng/ml is equivalent to 24 mIU/l.

Growth hormone (GH) plasma concentrations were determined by a solid-phase, two-site chemiluminescent enzyme immunometric (Immulite; Diagnostic Products Corp., Los Angeles, CA) using the Immulite automated analyzer. It has been shown a good correlation between this technique and reference methods (immunoradiometric assay) (Costong et al., 1995). The assay sensitivity is reported to be 0.003 ng/ml. Intra-assay CV were 3.56% and 6.5% for low and high controls and interassay CV 5.7% and 6.1% for low and high controls, respectively.

Pharmacokinetics and Statistical Analysis. With regard to plasma concentrations of MDMA and MDA, the following parameters were determined: peak concentration (C_{max}), time taken to reach peak concentration (T_{max}), and area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) and from 0 to infinite (AUC_{total}). Pharmacokinetic parameters of MDMA and MDA including absorption and elimination half-life were calculated using a computer program (PKCALC) (Shumaker, 1986). Values from physiological variables and hormone concentrations were transformed to differences from baseline. The peak effect until 6 h (maximum absolute change from baseline values) and the 6-h area under the curve (AUC_{0-6}) of effects versus time calculated by the trapezoidal rule were determined for each variable. In some variables, when the peak effect of the drug condition was observed after the first 6 h, an additional analysis of peak and AUC from 6 to 10 h (AUC_{6-10}) was done (e.g., heart rate and pupillary diameter for amphetamine). These transformations were analyzed by a one-way repeated measures ANOVA with drug conditions as the factor. When ANOVA results showed significant differences between treatment conditions, post hoc multiple comparisons were performed using the Tukey's test. Differences associated with p values lower than .05 were considered to be statistically significant.

Results

Changes in blood pressure, heart rate, oral temperature, and pupillary diameter induced by the study drugs are shown in Table 1 and Figs. 1 and 2. All active conditions (MDMA 125, MDMA 75, AMPH 40) produced an increase in blood pressure and in heart rate as compared with placebo

TABLE 1
Statistical results of physiological evaluations

Variable		ANOVA (d.f. 3,21)		Tukey Multiple Comparison Test					
				Placebo			MDMA 125		MDMA 75
		F	*p* value	MDMA 125	MDMA 75	AMPH 40	MDMA 75	AMPH 40	AMPH 40
Physiological variables									
SBP	AUC	20.14	<0.0001	**	**	**	N.S.	N.S.	N.S.
	PEAK	30.33	<0.0001	**	**	**	N.S.	N.S.	N.S.
DBP	AUC	7.69	0.0012	**	N.S.	**	N.S.	N.S.	N.S.
	PEAK	17.71	<0.0001	**	**	**	N.S.	N.S.	N.S.
Heart rate	AUC	18.59	<0.0001	**	**	**	N.S.	N.S.	N.S.
	PEAK	14.88	<0.0001	**	**	**	N.S.	N.S.	N.S.
Temperature	AUC	1.16	0.3467						
	PEAK	1.04	0.3968						
Pupillary diameter	AUC	39.52	<0.0001	**	**	N.S.	N.S.	**	**
	PEAK	36.53	<0.0001	**	**	N.S.	N.S.	**	**
Neuroendocrine variables									
Cortisol	AUC	17.04	<0.0001	**	**	N.S.	N.S.	**	N.S.
	PEAK	13.81	<0.0001	**	**	N.S.	N.S.	**	N.S.
Prolactin	AUC	13.21	<0.0001	**	N.S.	N.S.	**	**	N.S.
	PEAK	10.18	0.0002	**	N.S.	N.S.	*	**	N.S.
GH	AUC	0.96	0.4301						
	PEAK	0.81	0.5006						

PEAK, peak effects 0–6 hours; F, ANOVA's *F* value. Tukey's test statistical significance: * $p < .05$; ** $p < .01$; N.S., not significant; blank, not done (ANOVA not significant).

(when considering the peak effects, AUC, or both), although differences among them were not statistically significant. The peak difference between MDMA 125 and placebo was 44 mm Hg for SBP and 25 mm Hg for DBP; between MDMA 75 and placebo, 32 mm Hg for SBP and 18 mm Hg for DBP; and between AMPH 40 and placebo, 41 mm Hg for SBP and 23 for DBP. The maximal increase in either SBP or DBP was found at 90 min after the administration of all active drugs. In heart rate, the peak difference between MDMA 125 and placebo was 30 beats/min, MDMA 75 and placebo 24 beats/min, and between AMPH 40 and placebo 25 beats/min. The maximal increase in heart rate was observed at 60 min after MDMA administration and at 8 h after AMPH administration. Diagnostic criteria of hypertension (systolic blood pressure > 140 and/or diastolic blood pressure > 90 mm Hg) were fulfilled by four subjects in each of the MDMA and in the AMPH 40 conditions. Diagnostic criteria of isolated systolic hypertension were met by three subjects in the MDMA 125 condition and by one in the MDMA 75 condition. Hypertensive episodes showed a mean duration of 2 h (range 0.5–3.5). On the other hand, three subjects met diagnostic criteria of sinus tachycardia (>100 beats/min), two after the use of 125 mg of MDMA and one after the administration of 75 mg. Tachycardia lasted between 15 and 30 min.

Although temperature slightly increased during sessions when active drug conditions were given, no statistically significant differences were observed as compared with placebo. In relation to pupillary diameter, both doses of MDMA produced significant mydriasis as compared with placebo and with AMPH 40 in both AUC and peak effects (0–6 h). Peak differences between MDMA 125 and MDMA 75 in relation to placebo were 3.5 and 3 mm, respectively, and in relation to AMPH 40 were 2.5 and 2 mm, respectively. Maximal changes after MDMA administration were observed between 1 and 2 h. Otherwise, AMPH 40 induced a significant increase in pupil size compared with placebo at 10 h after drug administration, with a peak effect of 1 mm.

Neuroendocrine effects after the administration of MDMA 125, MDMA 75, AMPH 40, and placebo are showed in Table 1 and Fig. 3. Plasma cortisol concentrations were significantly higher (peak and AUC) after the administration of both MDMA conditions as compared with placebo. MDMA 125 showed also significant differences in comparison with AMPH 40. The peak difference in the plasma cortisol concentration between MDMA 125 and placebo was 23 μg/dl, between MDMA 75 and placebo 16 μg/dl, and between MDMA 125 and AMPH 40 15 μg/dl. Cortisol concentrations peaked at 2 h after MDMA administration and at 60 min of AMPH 40 ingestion. Plasma concentrations of prolactin were significantly higher (peak and AUC) after the use of MDMA 125 than after the administration of placebo, AMPH 40, and MDMA 75. Peak difference between MDMA 125 and placebo was 19 ng/ml, between MDMA 125 and MDMA 75 14 ng/ml, and between MDMA 125 and AMPH 40 19 ng/ml. Plasma prolactin concentrations peaked at 2 h after MDMA administration. GH plasma concentrations were not significantly influenced by any drug condition.

Time course of plasma concentrations of MDMA and MDA are presented in Fig. 4. Experimental (C_{max}, T_{max}, and AUC_{0-24}) and calculated pharmacokinetic parameters for MDMA and MDA at the two doses assayed are presented in Table 2. It should be noted that one of the subjects presented plasma concentrations much lower than the other participants especially after the administration of the 75-mg MDMA dose. His AUC values ranged between ¼ and ⅛ of those observed with the other volunteers. His calculated pharmacokinetic parameters showed also great differences, particularly plasma clearance, volume of distribution, and elimination half-life. Because no explanation has been found for this variation, the values could be considered outliers. For this reason, three mean values are reported in Table 2. The first mean value corresponds to all participating subjects in the clinical trial ($n = 8$); the next, after exclusion of the above-mentioned subject ($n = 7$); and the third corresponds to individual values estimated for the outlier subject. With regard to MDMA, T_{max} was observed at 2 h for both doses

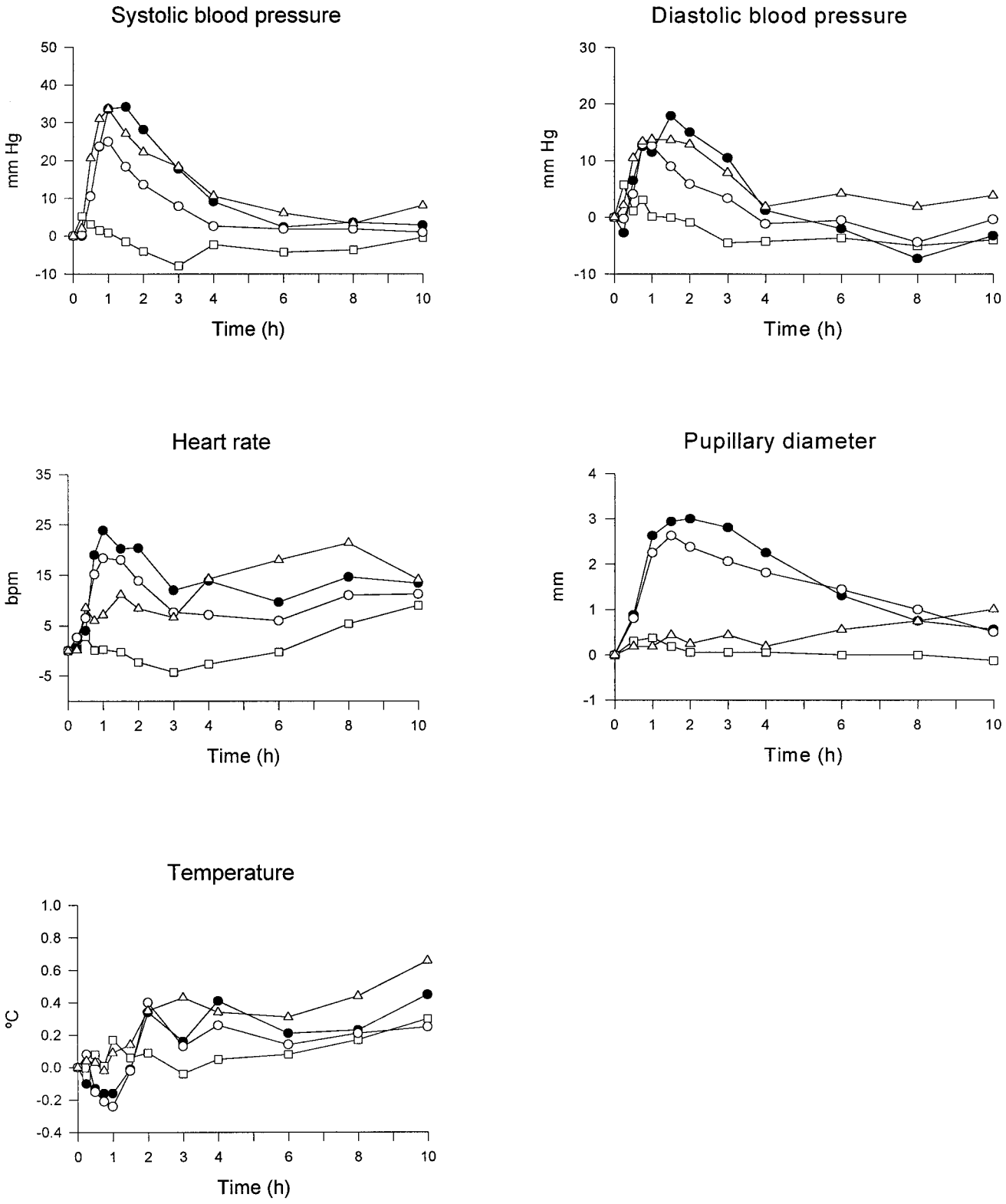

Fig. 1. Time course of drug effects on cardiovascular measures, temperature, and pupillary diameter (differences from baseline). Data points represent means from eight subjects. ●, 125 mg of MDMA; ○, 75 mg of MDMA; △, 40 mg of amphetamine; □, placebo.

studied. Plasma levels declined following a mono-exponential model. Mean elimination half-life (including all subjects) was 7.9 and 8.7 h after the 75-mg and 125-mg doses, respectively.

Plasma concentrations of MDA appeared slowly after MDMA administration. C_{max} of 13.7 and 7.8 ng/l for the MDMA 125-mg and 75-mg doses were reached at 5 to 7 h after administration. In reference to MDA pharmacokinetic, the MDA formation rate constant has been estimated for both MDMA doses of being about 0.75 h^{-1}. Elimination half-life of MDA was in a range of 16 to 28 h. Amphetamine

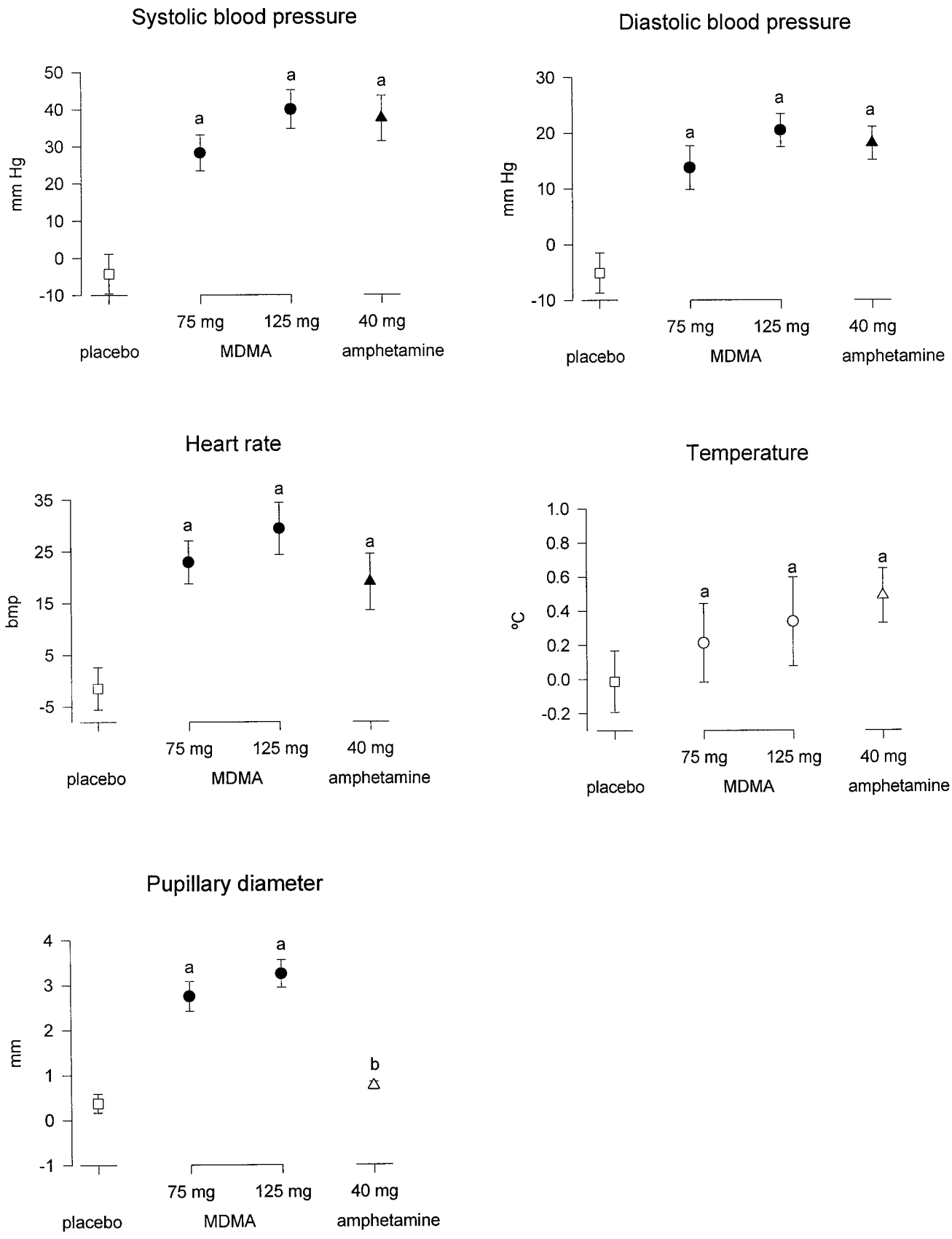

Fig. 2. Dose-response during peak drug effects (differences from baseline) on cardiovascular measures, temperature, and pupillary diameter. Data points represent means from eight subjects (± S.E.). Filled symbols indicate a significant difference from placebo ($p < .05$). Letters a and b indicate comparisons among the three active conditions; within the same panel, any two means designated with the same letter are not significantly different from each other at $p < .05$ (Tukey's post hoc test). ○, MDMA; △, amphetamine; □, placebo.

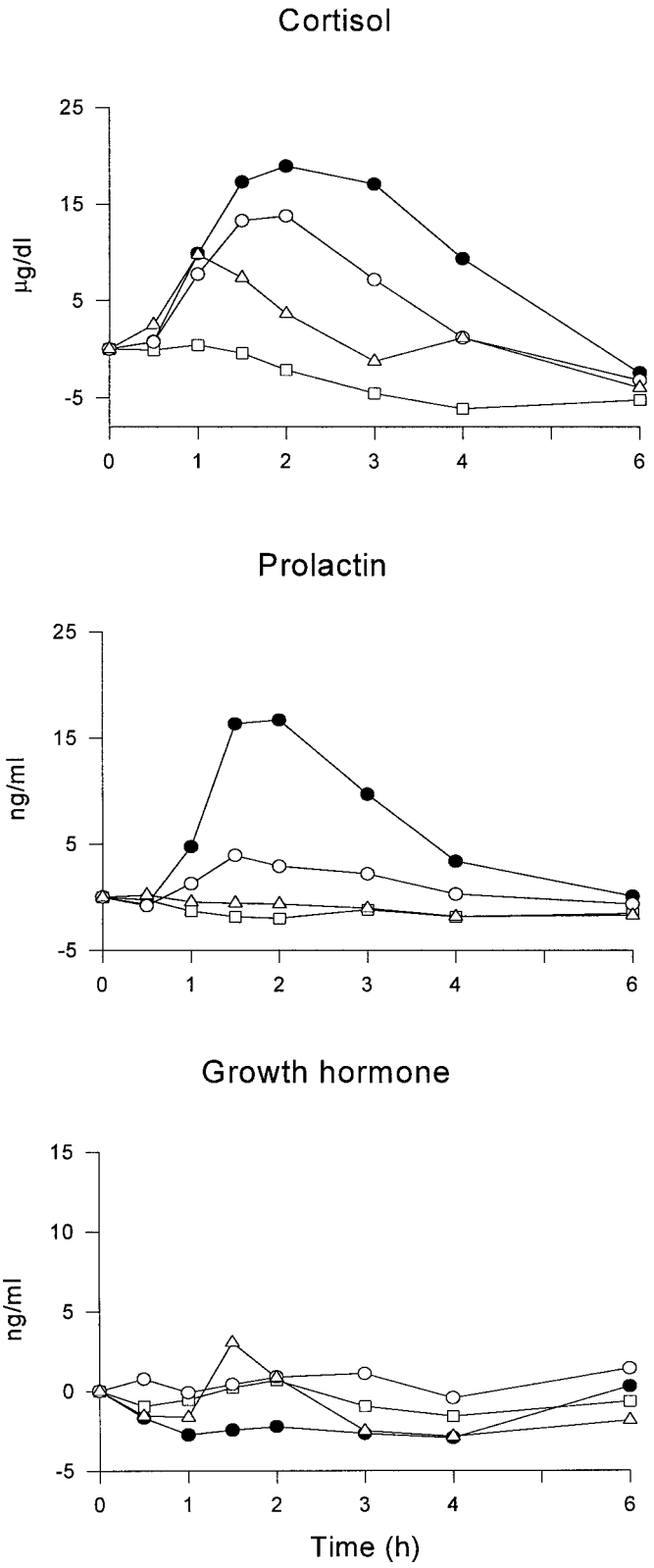

Fig. 3. Time course of drug effects on neuroendocrine measures (differences from baseline) (n = 8). Other details of the figure are similar to those for Fig. 1.

plasma concentrations are shown in Fig. 5. The T_{max} was 2 h and C_{max} 65 ng/ml. Its calculated elimination half-life was 15.3 h (range 9.5–22.5 h).

Discussion

MDMA administration produced marked increases in SBP and heart rate. These findings were also found when MDMA was administered at doses between 0.25 and 1 mg/kg (Grob et al., 1996) or 1.7 mg/kg (Vollenweider et al., 1998). A related substance, 3,4-methylenedioxyethylamphetamine (MDEA, 140 mg), produced a similar response pattern for SBP and heart rate but a lower increase for diastolic pressure (Gouzoulis et al., 1993). Hypertension and tachycardia have also been reported after MDMA intoxication (Henry et al., 1992). Although MDMA 125 produced a higher increase on cardiovascular parameters than MDMA 75, no statistically significant differences were found. Amphetamine produced a similar rise in blood pressure but a delayed response in heart rate. This time course response in heart rate for amphetamine seems to be due to a baroreceptive reflex bradycardia that occurs when a certain increase in blood pressure is reached (Martin et al., 1971; Jasinski and Preston, 1986; Brauer et al., 1996). Other stimulants, such as MDEA (Gouzoulis et al., 1993) and methylphenidate (Martin et al., 1971) have shown a response pattern similar to MDMA. The cardiovascular effects are attributable to the sympathomimetic properties of these drugs.

No significant changes in oral temperature were observed, although there was a tendency to rise throughout the study period, particularly with AMPH 40. These results are consistent with those of Grob et al. (1996) and Vollenweider et al. (1998) after MDMA administration and those of de Wit et al. (1997) after D-amphetamine administration. In experimental animals, the laboratory conditions may influence the pharmacological response to stimulants. When animals are tested in groups as compared with those tested individually, the toxicity of D-amphetamine is enhanced with an increase in body temperature and locomotor activity ("aggregate toxicity"). In humans, this phenomenon has been partially observed by De Wit et al. (1997). Higher increases in body temperature were registered when subjects were tested in groups than in individual testing conditions; the administration of amphetamine, however, did not influence the results. In reference to MDMA, the lethal dose—50 in male albino mice—was almost 5 times lower in aggregated than in isolated conditions (Davis et al., 1987). Hyperthermia has been reported in cases of MDMA intoxication (Dowling et al., 1987; Henry et al., 1992). The setting, laboratory versus natural (crowded conditions, physical exercise, dehydration, high environment temperature), may explain in part our observation of no significant changes in oral temperature.

Both MDMA doses induced similar mydriasis, whereas the effect on pupils produced by amphetamine was of lesser magnitude and showed a rather long latency period. These findings are consistent with those observed by other authors either with MDMA, in a noncontrolled study (Downing, 1986), or D-amphetamine (Jasinski and Preston, 1986). Mydriasis is also a common feature of MDMA intoxication (Dowling et al., 1986; Henry et al., 1992).

Although subjective effects produced by MDMA and AMPH 40 are not reported here, both drugs produced increases in scales related to euphoria and well being that were maximal after MDMA 125. Only MDMA produced mild changes in body perceptions (visual and auditory). None of the active drugs produced hallucinations. Amphetamine was correctly identified

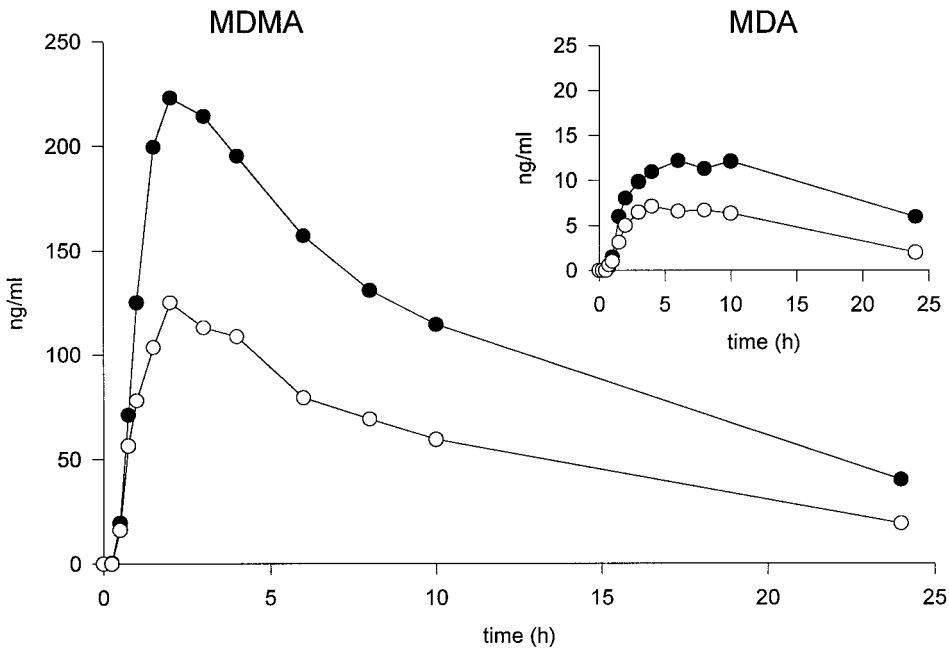

Fig. 4. Plasma concentration-time curve for MDMA and MDA (n = 8). ●, 125 mg of MDMA; ○, 75 mg of MDMA.

TABLE 2
Pharmacokinetic parameters for MDMA and MDA

		C_{max}	T_{max}	AUC_{0-24}	AUC_{total}[a]	k_a[b]	k_e	$T_{1/2a}$[c]	$T_{1/2}$
		ng/ml	*h*	*ng/ml · h^{-1}*	*ng/ml · h^{-1}*	*h^{-1}*	*h^{-1}*	*h*	*h*
MDMA (125 mg)									
n = 8[d]	Mean	236.4	2.4	2623.7	3190.7	2.1253	0.0923	0.41	8.73
	±S.D.	57.97	0.98	572.90	953.24	1.1001	0.0428	0.22	3.29
n = 7[d]	Mean	245.7	2.2	2745.5	3389.6	2.3136	0.0793	0.35	9.44
	±S.D.	55.75	0.81	494.31	831.10	1.0396	0.0238	0.13	2.83
n = 1[d]		171.1	4	1770.7	1789.1	0.8066	0.1832	0.86	3.78
MDMA (75 mg)									
n = 8	Mean	130.9	1.8	1331.5	1541.6	2.3835	0.1171	0.42	7.86
	±S.D.	38.60	0.38	646.03	721.56	2.1362	0.0818	0.20	3.58
n = 7	mean	141.6	1.7	1484.5	1720.8	2.5734	0.0910	0.39	8.66
	±S.D.	25.79	0.39	518.16	554.79	2.2332	0.0380	0.18	3.01
n = 1		55.9	2	260.7	287.4	1.0542	0.2999	0.66	2.31
MDA (125 mg)									
n = 8	mean	13.7	7.1	215.2		0.7715	0.0434	1.42	27.66
	±S.D.	1.56	2.80	68.46		0.8195	0.0264	0.73	25.98
n = 7	mean	14.2	7.3	235.5		0.7864	0.0398	1.47	30.17
	±S.D.	0.96	2.98	40.40		0.8840	0.0263	0.77	26.99
n = 1		10.5	6.0	73.3		0.6671	0.0687	1.04	10.09
MDA (75 mg)									
n = 8	Mean	7.8	5.1	122.3		0.7346	0.0502	1.23	16.11
	±S.D.	2.47	2.64	66.68		0.4370	0.0168	0.63	8.33
n = 7	mean	8.4	5.4	132.0		0.6073	0.0479	1.35	16.91
	±S.D.	1.84	2.70	65.63		0.2677	0.0167	0.58	8.66
n = 1		3.4	3.0	54.3		1.6254	0.0663	0.43	10.45

k_a, absorption constant; k_e, elimination constant; $T_{1/2a}$, absorption half-life; $T_{1/2}$, elimination half-life.
[a] Not calculated for MDA.
[b] Formation constant rate of MDA.
[c] Formation half-life of MDA.
[d] n = 8, all subjects; n = 7, all subjects excluding one considered an outlier; n = 1, subject considered an outlier (see text for explanations).

by all but two subjects, and one subject identified MDMA 125 as amphetamine.

In relation to the neuroendocrine effects, only MDMA produced significant increases in plasma cortisol and prolactin concentrations. These results are consistent with those of Grob et al. (1996) who reported increased adrenocorticotropin and prolactin concentrations after use of 0.75 to 1 mg/kg of MDMA. Although cortisol increases are consistent with activation of serotonergic neurotransmission, dopaminergic and noradrenergic mechanisms may also be involved. The mechanism of action of MDMA is poorly understood. Serotonergic effects due to 5-HT release and/or inhibition of 5-HT uptake are prominent, but the release of other monoamines, such as dopamine and norepinephrine, has also been demonstrated (Steele et al., 1994; White et al., 1996). In rats, corticosterone increased after MDMA administration (Nash et al., 1988). 5-HT_2 antagonists (ketanserin and mianserin) and a 5-HT uptake inhibitor (fluoxetine) produced a statistically significant inhibition of MDMA-induced corticosterone secretion. The administration of 5-HT_{1A} agonists (buspirone, gepirone) was followed by marked increases in adrenocorticotropin and cortisol (Anderson et al., 1990; Meltzer and

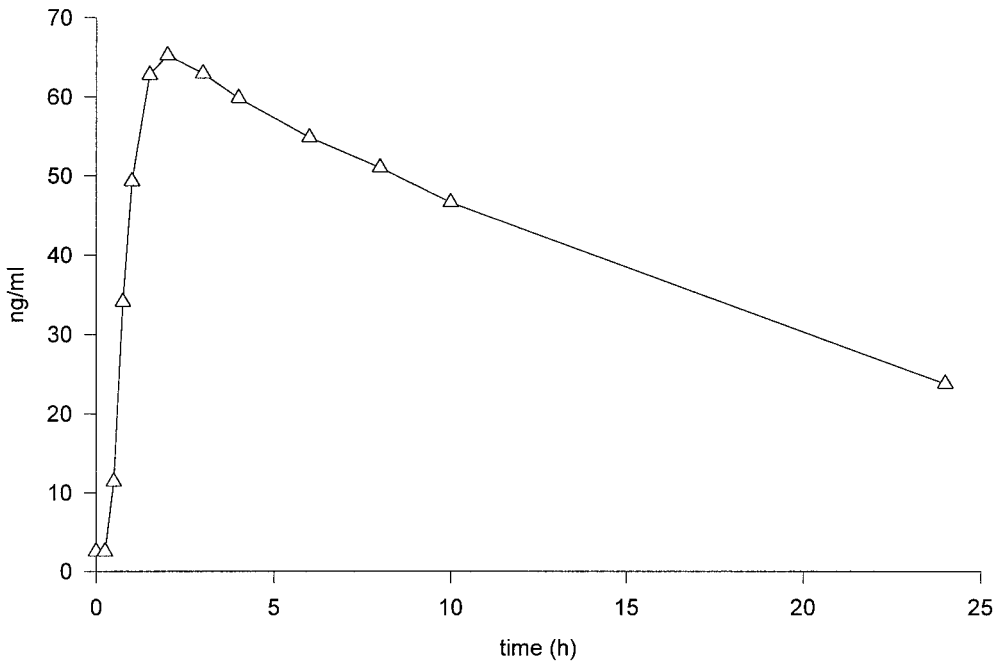

Fig. 5. Plasma concentration-time curve of amphetamine (n = 8).

Maes, 1994). Fenfluramine, a serotonergic drug, also produced a dose-related increase in concentrations of cortisol and prolactin (Nurnberger et al., 1984). It should be noted that fenfluramine, dexfenfluramine, and MDMA have shown neurotoxic effects on serotonergic axons in animal studies. In humans, MDEA increased cortisol and prolactin levels (Gouzoulis et al., 1993). Amphetamine seems to act predominantly in dopaminergic and noradrenergic systems but with some serotonergic properties. We found a slight increase in plasma cortisol concentrations after the use of amphetamine, whereas others (Nurnberger et al., 1984) have shown a marked increase in cortisol levels after i.v. dextroamphetamine administration.

Only MDMA 125 induced an increase in prolactin levels. Prolactin secretion is mainly mediated by dopaminergic and serotonergic systems. In rats, MDMA administration increased prolactin concentrations (Nash et al., 1988). Serotonin antagonists (ketanserin, mianserin, cyproheptadine, and metergoline) did not produce changes in MDMA-induced prolactin secretion, but pretreatment with *p*-chlorophenylalanine that depleted cortical and hypothalamic 5-HT and 5-hydroxyindole acetic acid significantly blunted MDMA-induced prolactin secretion. In humans, buspirone and gepirone, both 5-HT_{1A} agonists, raised prolactin levels (Anderson et al., 1990; Meltzer and Maes, 1994). As mentioned previously, both fenfluramine (Sommers et al., 1994; Yatham, 1996) and MDEA (Gouzoulis et al., 1993) increased prolactin concentrations. With regard to amphetamine, both increases or decreases have been described (Dommisse et al., 1984; Nurnberger et al., 1984). No changes after amphetamine administration were noticed in the present study. The interrelation between serotonin and dopamine systems, with the existence of serotonin terminations influencing dopamine neurons, could explain in part our findings (Rittenhouse et al., 1993).

Neither MDMA nor amphetamine induced changes in GH. Different 5-HT_{1A} agonists could produce increases in GH levels (Anderson et al., 1990; Meltzer and Maes, 1994). Previous reports have shown an increase in GH concentrations after the administration of high doses of dextroamphetamine or when the drug was administered i.v. (Dommisse et al., 1984; Nurnberger et al., 1984). GH concentrations also remained unchanged after the administration of MDEA (Gouzoulis et al., 1993).

Blood concentration profile and pharmacokinetic parameters of amphetamine were similar to those described by others (Jenkins and Cone, 1998). To our knowledge, this is the first complete description of the pharmacokinetics of MDMA and its metabolites after the administration of two doses to a considerable number of subjects in the range of doses used for recreational purposes. The T_{max} was attained at 2 h, a similar result as reported by Helmlin et al. (1996) and Verebey et al. (1988), although it was reached at 4 h in the study of Henry et al. (1998). Peak concentrations, taking into account the proportions between doses, are also in agreement with the mentioned previous findings. The elimination half-life of 125 and 75 mg of MDMA was about 8 to 9 h, similar to that reported after 50 mg (7.6 h) (Verebey et al., 1988). These values are lower than those reported for methamphetamine (10–12 h) or amphetamine (12–15 h in literature, and 15.3 h in our results) (Jenkins and Cone, 1998).

Although the increase in the dose accounted for a factor of 1.66 (from 75 to 125 mg), an increase was observed in the AUC_{total} and C_{max} of 2 and 1.8, respectively. A possible nonlinear pharmacokinetics could be suggested when considering together this result and those of the pilot studies. After the administration of 150 mg to two volunteers, a disproportional increase in MDMA plasma concentrations was observed (de la Torre et al., 1999). The possible nonlinear kinetics should be confirmed in studies with larger samples.

MDA, formed by *N*-demethylation of MDMA, seems to be a minor metabolite, representing 8 to 9% of the concentrations of MDMA (AUC comparisons). This finding is further supported by the fact that MDA urinary recovery is about 1% of the dose administered (data not shown), whereas for methamphetamine the *N*-demethylated product (amphetamine) is about 10% (Mendelson et al., 1995).

The pharmacokinetic parameters observed in one of our subjects could be considered as outlier values. Plasma levels after the 75-mg dose were 4 times lower than those observed in the remaining participants. The concentrations after 125 mg of MDMA and amphetamine were also the lowest found in any subject but near to the values observed in other volunteers. AUC_{0-24} for 75-mg and 125-mg MDMA doses were 17% and 64% of the mean values, respectively. In relation to pharmacodynamic measures, after the 75-mg dose, this subject did not report the appearance of any subjective effect, but mild increases in blood pressure and heart rate were recorded. Responses after 125 mg of MDMA and amphetamine had values in the range observed by other participants. One possible explanation could be a low bioavailability, but individual data of urinary recovery of MDMA and metabolites were similar to the rest of the participants (data not shown). This subject had much higher concentrations of 4-hydroxy-3-methoxymethamphetamine in urine, a major MDMA metabolite, when compared with other subjects (data not shown). However, a conclusive explanation for this finding cannot be provided.

The time course of blood concentrations of MDMA and its pharmacological effects rise and fall with a similar profile. Drug concentrations increased, and parallel increases in physiologic and hormonal measures were observed. Both peak concentrations and peak effects were obtained between 1 and 2 h and decreased to return to baseline values 4 to 6 h after drug administration.

In summary, MDMA given at recreational doses produced mydriasis and marked increases in blood pressure, heart rate, and plasma cortisol and prolactin concentrations. Its elimination half-life was about 8 to 9 h. According to these findings obtained in the laboratory setting, MDMA consumption in crowded conditions, high ambient temperature, and physical activity ("rave parties") may be associated with a potential life-threatening increase in the cardiovascular toxicity of the drug.

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