

Plasma Drug Concentrations and Physiological Measures in 'Dance Party' Participants

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The increasing use of (\pm) 3,4-methylenedioxymethamphetamine (MDMA) in the setting of large dance parties ('raves') and clubs has been the source of some concern, because of potential acute adverse events, and because animal studies suggest that MDMA has the potential to damage brain serotonin (5-HT) neurons. However, it is not yet known whether MDMA, as used in the setting of dance parties, leads to plasma levels of MDMA that are associated with toxicity to 5-HT neurons in animals. The present study sought to address this question. Plasma MDMA concentrations, vital signs, and a variety of blood and urine measures were obtained prior to, and hours after, individuals attended a dance party. After the dance party, subjects were without clinical complaints, had measurable amounts of residual MDMA in plasma, and nearly half of the subjects also tested positive for methamphetamine, another amphetamine analog that has been shown to have 5-HT neurotoxic potential in animals. Plasma concentrations of MDMA did not correlate with self-reported use of 'ecstasy' and, in some subjects, overlapped with those that have been associated with 5-HT neurotoxicity in non-human primates. Additional subjects were likely to have had similar concentrations while at the dance party, when one considers the reported time of drug ingestion and the plasma half-life of MDMA in humans. Hematological and biochemical analyses were generally unremarkable. Moderate increases in blood pressure, heart rate and body temperature were observed in the subjects with the highest MDMA plasma concentrations. These findings are consistent with epidemiological findings that most people who use MDMA at dance parties do not develop serious clinical complications, and suggest that some of these individuals may be at risk for developing MDMA-induced toxicity to brain serotonin neurons.

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INTRODUCTION

The recreational use of 'party drugs' is a growing fashion with young people in many countries throughout the world (Kalant, 2001; Green *et al*, 2003). Of these drugs, 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') is the most popular and has received particular attention because, on rare occasions, its use has led to fatalities (Schifano *et al*, 2003; Gowing *et al*, 2002), and because, in animals, it has been shown to have the potential to lead to a persistent loss of brain serotonin (5-HT) axonal markers (Steele *et al*, 1994; Ricaurte *et al*, 2000; Green *et al*, 2003). 'Ecstasy' is normally taken as a pill and in the strictest sense only contains MDMA.

Despite concerns about MDMA, there is a paucity of data that directly address the relevance of animal studies demonstrating MDMA's neurotoxic potential toward brain 5-HT neurons to human recreational 'ecstasy' users. In particular, there are no data available regarding plasma levels of MDMA in recreational 'ecstasy' consumers who do not develop medical complications (or are not apprehended by authorities). There have been several studies in which MDMA was administered to humans in a controlled laboratory setting and in which plasma pharmacokinetics of MDMA were measured (Helmlin *et al*, 1996; de la Torre *et al*, 2000, 2004; Pacifici *et al*, 2001, 2002). However, these studies necessarily involved a limited dose range that was well below that reported by some MDMA users (Parrott, 2005). Further, it is possible that factors associated with MDMA use in dance settings (eg increased temperature, crowding, activity, ingestion of other psychoactive substances) may influence the pharmacokinetics, pharmacodynamics, and/or neurotoxic potential of MDMA.

The public and scientific debate surrounding MDMA and acute adverse effects has highlighted the low incidence of clinical presentations demonstrating severe adverse effects when one considers the very large number of users of this

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drug (Topp *et al*, 1999; Green *et al*, 2003). It has also raised questions about the relevance of animal data to the effects of the drug in humans, and has underscored the lack of data in humans using doses and conditions that approximate the recreational use of the drug. The debate on the possibility of long-term neurotoxicity as a result of MDMA use has largely centered on whether doses and dosage regimens used in animal models reflect those achieved by recreational drug users (Hegadoren *et al*, 1999). Interpretation of the animal data from experiments in animals would be greatly facilitated if we had information on the blood concentrations of MDMA in recreational users of these drugs in a normal use situation. This would allow direct comparison between animals (Colado *et al*, 1995; Bowyer *et al*, 2003) and humans regarding blood concentration–effect relationships for MDMA.

To address some of these issues, we have undertaken a study in young persons who frequent ‘raves’ and are users of ‘ecstasy’. Individuals planning to attend a dance party were evaluated before, and several hours after the party. Outcome measures included vital signs, blood chemistry panels, hematologic measures, urine electrolytes and osmolality, and urine and blood screens for illicit drugs.

METHODS

Subjects

A total of 27 subjects of both genders were recruited by word of mouth on the basis that they were regular attendees at ‘raves’ and had previously taken ‘ecstasy’ at least 5 times. Ethics approval for the study was granted by the Royal Adelaide Hospital Human Ethics Committee and all subjects provided informed consent. The information sheet clearly stated that the use of drugs during the study day was not a condition of acceptance into the study.

Procedures

On the evening prior to the dance party held in South Australia subjects completed a questionnaire on their prior drug use. Peripheral venous blood and urine samples were taken for analysis. Tympanic temperature was recorded by a clinical infrared thermometer (Braun Kronberg, Germany). Resting blood pressure was recorded by sphygmomanometry and pulse rate was measured. The subjects were then allowed to proceed with their normal activities at the dance party. At the mid-point of their dance party (0400 hour) their tympanic temperature was taken at the dance venue. At the end of the party (0800–1000 hours), usually while at a ‘recovery’ or ‘after’ party, further blood and urine sample were collected and subjects were asked to fill in a questionnaire on their experiences over the past 12 h. Their tympanic temperature, resting blood pressure, and pulse rate were measured again, after at least 10 min of rest.

Blood and Urine Analysis

Standard hematological and biochemical blood examinations were performed and serum and urine electrolytes and osmolality were measured by a certified clinical pathology laboratory. Plasma arginine vasopressin (antidiuretic hor-

mone) concentrations were measured by radioimmunoassay (Buhlmann RK-AR1, Allischwil, Switzerland).

Drug Analyses

Initial drug screens, using commercially available enzyme-linked immunosorbent assay kits (ELISA), were performed on all plasma samples to determine the presence of illicit drugs. Amphetamines were detected with the Amphetamines Kit (International Diagnostic Systems, St Joseph, MI). This assay has sufficient sensitivity to detect concentrations of 0.1 mg/l of methylamphetamine. Opiates, methadone, benzodiazepines, and cannabinoids were detected with the SINGLESTEP™ kit (Diagnostix, Ltd, Ontario, Canada). The sensitivity of these assays were 0.02 mg/l for opiates, 0.02 mg/l for methadone, 0.05 mg/l for benzodiazepines, and 0.005 mg/l for cannabinoids.

Positive samples were further analyzed by gas chromatography using the following method. Plasma samples were extracted with butyl chloride after addition of 0.2 N NaOH. The solvent extract was evaporated at 40°C using dry N₂ and reconstituted in 100 µl of ethanol. Amphetamines measured using Agilent 5890 series II Plus gas chromatography equipped with dual nitrogen-phosphorus detectors and a 7673A auto-sampler. The columns consisted of a 5 µm phenyl-methyl deactivated guard column (0.53 mm i.d.) split equally to a DB-1 capillary column (10 m × 0.32 mm i.d.) and a DB-17 capillary column (10 m × 0.32 mm i.d.). The oven was held at 100°C for 0.5 min and then ramped at 10°C/min to 280°C and held for 8.5 min. The injection port temperature was 250°C and the temperature of the detectors was 280°C. The analysis was capable of detecting a large range of licit and illicit basic drugs including amphetamine, methylamphetamine, phentermine, mephentermine, paramethoxy-amphetamine (PMA), methylenedioxymethylamphetamine (MDMA), methylenedioxyamphetamine (MDA), ephedrine and pseudoephedrine. The limit of detection for these substances was 0.01 mg/l in all cases. The presence of moclobemide in one sample was confirmed by gas chromatography with mass selective detector (Agilent 5972 GC/MSD). The column used was a HP5MS capillary (15 m × 0.25 mm i.d.) using a similar temperature program as the amphetamine screen.

Gamma hydroxybutyrate (GHB) was measured after conversion to the gamma-butyrolactone by the addition of sulfuric acid to the plasma and extraction with methylene chloride. After concentration of the extract at 40°C using dry N₂, it was analyzed by capillary GC (HP5MS 15 m × 0.25 mm i.d.) operated at 60°C with detection by mass selective detector (Agilent 5972 hGC/MSD).

Statistical Analysis

Data sets had an *n* of 27 with the exception of the drug histories (*n* = 25, Table 1), blood exam data (*n* = 24–26, Table 2), and self-reported data (*n* = 23–25). The missing data were samples not collected due to lack of compliance or availability of the participant at that time.

Paired *t*-tests or repeated measures ANOVA were undertaken to compare measures before, during and after drug intake. Pearson’s correlations were performed and *p* < 0.05 taken as significant.

RESULTS

The mean age of subjects was 26.3 ± 3.6 years and 56% were male. All of them reported regular attendance at 'dance parties' where they usually used illicit drugs. Their drug use history is shown in Table 1 and indicates polydrug use. All subjects regularly used alcohol and MDMA and most used methamphetamine and marijuana; LSD, nitrous oxide, tobacco, cocaine, and GHB were used to a lesser extent. Very few of the subjects used opioids and, among those who did, the use was very infrequent. The subjects as a group used MDMA on 2.5 ± 3.2 days per month with a range of 1–12.

Self-report data on drug use, exertion, and fluid intake on the study night indicated that the subjects took between 1 and 7 (mean 2.5 ± 1.4) pills during the study period. In all, 18 subjects (72%) reported low exertion levels, two (8%) with medium, and five (20%) with high exertion levels. Eight subjects (35%) reported low fluid intake, 14 (61%) with medium, and one (4%) with high fluid intake. All subjects had achieved desirable drug effects during the study period. At the time of the final blood sample, five subjects (22%) reported no subjective effects of the drug, while 12 subjects (52%) reported mild effects, and six (26%) reported strong effects.

Blood drug screens on the morning blood samples indicated that all subjects were positive for MDMA and of these, 11 (41%) had also ingested methamphetamine and nine (33%) THC. Other drugs detected were benzodiazepines (three subjects), moclobemide (one subject), and pseudoephedrine (one subject).

The data from quantitative analysis of blood samples for MDMA and methamphetamine are shown in Figure 1. There was a wide range of concentrations of MDMA measured (mean 0.31 ± 0.21 mg/l, $n=27$). Five of the subjects displayed very high MDMA concentrations that would be classified as toxic to lethal by forensic laboratory guidelines (Odell and Drummer, 2001; Baselt, 2002), which overlap concentrations of MDMA that have been associated with neurotoxicity in monkeys (Mechan *et al*, 2006, same issue). Mean methamphetamine concentration was 0.16 ± 0.27 mg/l ($n=11$). One individual had an extremely high plasma concentration of methamphetamine.

Table 1 Drug History of the Subjects in this Study

Drug	% Ever used (n)	Frequency of use (days/month) mean \pm SD	Range
Alcohol	100% (25)	11.5 ± 8.9	1–30
MDMA	100% (25)	2.5 ± 2.3	1–12
Methamphetamine	96% (24)	5.9 ± 8.4	0–30
Marijuana	96% (23)	5.9 ± 10.4	0–30
LSD	84% (21)	1.1 ± 1.6	0–5
Inhalants	79% (19)	0.5 ± 0.8	0–2
Tobacco	75% (18)	15.9 ± 15.0	0–31
Cocaine	63% (15)	0.6 ± 0.8	0–2
GHB	48% (12)	3.1 ± 6.6	0–20
Opiates	13% (3)	0	0

Data are expressed as the percentage of subjects who have ever used the drug in question and frequency of use per month in days.

Cardiovascular measures and body temperature data are shown in Figure 2. There were small but significant increases in heart rate ($p=0.003$, $n=23$), systolic ($p=0.019$, $n=23$), and diastolic ($p=0.004$, $n=23$) blood

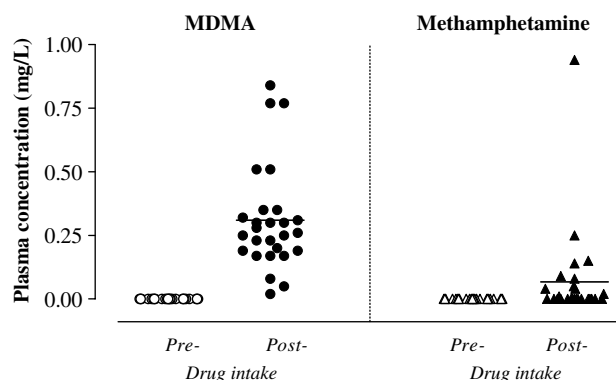


Figure 1 Plasma concentrations of MDMA and methamphetamine pre- and post-drug intake ($n=27$). The horizontal lines indicate the group mean.

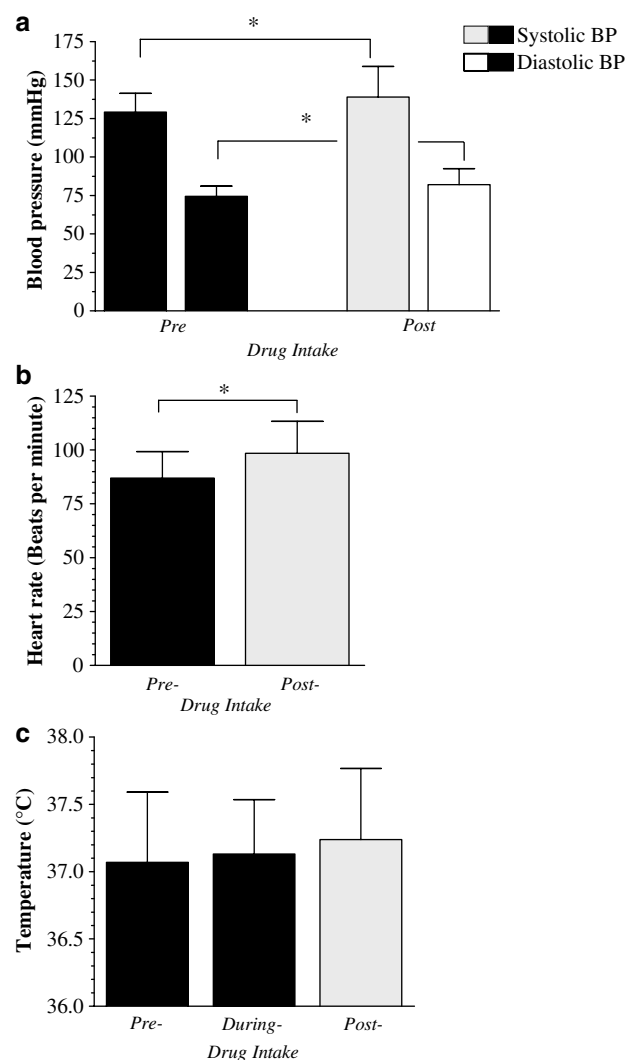


Figure 2 Cardiovascular and temperature effects after drug intake. Data are represented as mean \pm SD. (a) Changes in systolic ($p=0.019$) and diastolic blood pressure ($p=0.004$). (b) Changes in heart rate after drug intake ($p=0.003$). (c) Body temperature changes.

Table 2 Changes in Full Blood Exam Parameters, prior to and after Drug Intake

	Pre-/post-drug intake	Full blood exam		Subjects outside normal range	
		Mean	SD (n)	Over (%)	Under (%)
Haemoglobin (g/l)	Pre	147.7	12.4 (24)	0	0
	Post	148.3	14.3 (26)	0	1 (4%)
Packed cell volume (L/l)	Pre	0.43	0.03 (24)	0	0
	Post	0.43	0.04 (26)	0	1 (4%)
Platelets ($10^9/l$)	Pre	263.6	36 (24)	0	0
	Post	271.0	42 (26)	0	0
Neutrophil ($10^9/l$)	Pre	4.9*	1.5 (24)	2 (8%)	0
	Post	7.1	1.9 (26)	10 (38%)	0
Monocyte ($10^9/l$)	Pre	0.48	0.22 (24)	1 (4%)	0
	Post	0.43	0.21 (26)	2 (8%)	3 (12%)
Lymphocyte ($10^9/l$)	Pre	2.6*	0.56 (24)	1 (4%)	0
	Post	1.62	0.56 (26)	0	5 (19%)
Eosinophil ($10^9/l$)	Pre	0.40	1.13 (24)	1 (4%)	0
	Post	0.08	0.25 (26)	1 (4%)	12 (46%)
Basophil ($10^9/l$)	Pre	0.04	0.03 (24)	0	0
	Post	0.02	0.03 (26)	0	0
White cell ($10^9/l$)	Pre	8.42	2.3 (24)	3 (13%)	0
	Post	9.27	2.1 (26)	5 (19%)	0

Pre- and post-drug intake values were compared using paired *t*-tests with Bonferroni correction for multiple comparisons, * indicates $p < 0.001$. Subjects with blood concentrations above or below the normal reference range for each parameter are presented. Mean data are all within the normal range.

pressures between pre and post-drug times. There was a small progressive increase in temperature during and after drug use, which did not reach statistical significance. A modest correlation ($r = 0.41$, $p = 0.037$, 95% CI = 0.027–0.69, $n = 26$) between temperature and combined methamphetamine and MDMA concentrations (ie concentration in g/l of methamphetamine plus concentration in g/l of MDMA) was observed. There were no other significant correlations between drug use or concentrations and cardiovascular or temperature changes. The only other correlation that came close to significance was for plasma MDMA concentration and temperature ($r = 0.38$, $p = 0.058$, 95% CI = 0.01–0.6, $n = 26$). Correlations for cardiovascular parameters and drug concentrations were all below 0.3.

The hematological data (Table 2) indicated that some of the predrug values were outside the normal range. There was a significant increase in neutrophil count ($p < 0.0001$, 95% CI = –3.14–1.53, $n = 23$) and a decrease in lymphocyte count when pre- and post-drug values were compared ($p < 0.0001$, 95% CI = 0.74–1.30, $n = 23$). These changes may be related to drug use or other factors such as exercise, although no correlation was found when exercise or drug concentrations were compared with the changes in white cells.

Blood biochemistry (not shown) indicated that some subjects had values outside the normal range prior to drug use. Generally, these were higher than normal and may reflect lifestyle, including regular MDMA and/or methamphetamine use. Comparisons between pre- and post-drug measures showed a significant difference for urea ($p < 0.0001$, 95% CI = 0.68–1.46, $n = 24$). Although no group effect was seen, one subject showed a very large

increase in plasma vasopressin concentrations after drug administration.

Urine biochemistry revealed no differences between pre- and post-drug values. However, these data were incomplete due to the difficulties experienced by subjects in voiding urine postdrug intake.

Table 3 shows the summarized postdrug data from the six subjects who demonstrated plasma methamphetamine or MDMA concentrations in the 'toxic' to 'lethal' range. Subject 1 had taken an unknown quantity of methamphetamine and MDMA. He had a low plasma concentration of MDMA but a high concentration of methamphetamine. He had elevated blood pressure but a normal body temperature. The remaining subjects in this group had high MDMA concentrations and three of them had detectable methamphetamine. The two males with the highest concentrations of MDMA also had elevated core temperatures. One of these (no. 5) also had a highly elevated plasma vasopressin of 27 pmol/l but did not display any other unusual blood biochemistry. With a serum sodium at 143 mmol/l there was no evidence of hyponatremia. It is notable that heart rate and blood pressure in this group were high when compared with normal age adjusted values, although these elevations could be related to recent activity at a dance party.

DISCUSSION

This is the first study in which MDMA plasma concentrations and clinical measures were obtained from humans who used 'ecstasy' in a normal recreational setting and did not come to the attention of medical personnel or legal

Table 3 Summarized Data from the Six Subjects with High Plasma MDMA or Methamphetamine Concentrations

Subject number (gender)	Blood drug concentration (mg/l)		Reported dose (no. pills)	Blood pressure (mmHg)		Heart rate (BPM)	Temperature (°C)
	MDMA	Methamphetamine		Systolic	Diastolic		
1 (M)	0.02	0.94	2	165	100	104	37.0
2 (F)	0.51	0.08	2.5	140	80	96	37.0
3 (M)	0.51	0	4	140	90	128	37.1
4 (F)	0.77	0	1	130	80	120	37.1
5 (M)	0.77	0.25	4	155	97	95	38.6
6 (M)	0.84	0.01	4	150	95	95	38.0

authorities. The major finding of the study was that, hours after drug ingestion, some recreational MDMA users had plasma concentrations of MDMA that exceeded the lower observed values for plasma concentrations associated with neurotoxicity in pre-clinical studies of MDMA (Mechan *et al*, 2006, same issue). Additional subjects were likely to have also achieved levels in the 'toxic range' during the dance party, after consideration of the plasma half-life of MDMA in humans (5.6–8.6 h, de la Torre *et al*, 2000; Kraemer and Maurer, 2002), along with the self-reported time of drug use. Notably, plasma levels in this range are considered to be in the toxic to lethal range by forensic laboratory guidelines (Odell and Drummer, 2001; Baselt, 2002), yet none of the subjects had clinical complaints. This observation suggests that the plasma concentration of MDMA, *per se*, is not a reliable predictor of acute MDMA-induced medical complications. Indeed, the present data provide evidence that repeated use of MDMA results in a tolerance to its sympathomimetic effects. Although there have been previous clinical accounts of diminished psychoactive effects of MDMA with repeated use (eg Scholey *et al*, 2004), tolerance to its other actions has not been established.

The present data underscore the strengths and weaknesses of controlled laboratory and preclinical studies in which MDMA is administered. Clearly, a strength of these studies is that potential confounding variables can be controlled, thereby permitting isolation of a particular experimental outcome, such as plasma MDMA concentration. On the other hand, with regard to clinical studies, if a goal is to model MDMA use in a recreational setting, this very strength could be viewed as a limitation, since as in the present study, humans often take a variety of other substances along with MDMA (sometimes unwittingly (Cole *et al*, 2002)), and since variables present at dance parties might influence the pharmacokinetics, pharmacodynamics, and toxicity of MDMA. Similarly, preclinical studies generally involve drug-naïve animals. The present data suggest that, over time, tolerance to the pharmacologic effects of MDMA takes place and, as such, preclinical studies may not provide the most appropriate model of MDMA use in a recreational setting, in which experienced users may gradually increase their dose of drug. Our results on plasma concentrations of MDMA in 'ecstasy' consumers who had attended a dance party are in line with those

contained in reports on individuals who presented to emergency rooms or came to the attention of authorities after ingesting ecstasy (Samyn *et al*, 2002; Peters *et al*, 2003; Lora-Tamayo *et al*, 2004).

Despite the obvious differences in experimental design, the plasma concentrations of MDMA measured in the majority of our subjects were comparable with those measured in the controlled clinical study of de la Torre *et al* (2000). In that study the highest dose administered was 150 mg (approx 2 mg/kg) to two subjects, but the paper stated that this dose was abandoned in subsequent studies because of adverse subjective and cardiovascular effects. The maximum plasma concentrations (C_{\max}) measured in those subjects were 441 and 487 ng/ml at 1.5–2 h after ingestion of a 150 mg dose. In our study we can assume that our samples were unlikely to have been taken at C_{\max} , but, even so, three of our subjects had blood levels well in excess of those reported by de la Torre *et al* (2000). In that study, and other studies, using doses of 75 and 100 mg of MDMA, C_{\max} figures of around 200–300 ng/ml were reported (de la Torre *et al*, 2000; Pacifici *et al*, 2001; Samyn *et al*, 2002). Therefore, it is evident that in a recreational setting some individuals achieve much higher blood concentrations of MDMA than those obtained in experiments with volunteers in controlled clinical studies. The higher blood concentrations seen here may be because of higher doses used in a recreational setting, but may also be because of the contribution of MDMA's known nonlinear pharmacokinetic profile. In particular, plasma concentrations of MDMA in humans and non-human primates increase disproportionately after single doses of increasing magnitude or with repeated doses of the same magnitude (de la Torre *et al*, 2000; Mechan *et al*, 2006, same issue).

Overall, there was no correlation between reported drug use and plasma drug concentrations. This suggests a wide intersubject variation in pharmacokinetics, although the timing of the drug sampling in this study was not identical for each subject. However, it does highlight the danger in using self report data as an indication of biological exposure to the drug, as has been done in field studies to date.

As noted above, the present data provide suggestive evidence for tolerance to MDMA's sympathomimetic/behavioral effects, since plasma levels in the 'toxic' range were well-tolerated by these experienced MDMA users. Controlled preclinical and clinical studies have previously

demonstrated tolerance to these effects of methamphetamine (eg Segal *et al*, 2003; Comer *et al*, 2001). Further, there is also evidence that animals treated with gradually increasing doses of methamphetamine develop a partial tolerance to the neurotoxic effects of methamphetamine toward brain dopamine and serotonin neurons (Schmidt *et al*, 1985; Gygi *et al*, 1996; Segal *et al*, 2003). Similar studies are not available for MDMA, but the present data suggesting marked behavioral tolerance to the effects of MDMA indicate that such studies are in order.

The biochemistry and hematology was generally unremarkable with a few exceptions, for which the biological significance is not clear. A decrease in plasma urea and lymphocytes were seen with an increase in neutrophils. It is of interest that a number of studies have indicated that MDMA may have an influence in suppressing immune function in humans due to depression of lymphocyte cell subsets such as T-helper cells and CD4 T-helper cells (Pacifi *et al*, 1999, 2002). The biological significance of the decreased urea is unclear and is contrary to what would be predicted from case studies demonstrating rhabdomyolysis and renal failure. It is possible that this individual increased his fluid intake in an effort to prevent rhabdomyolysis, as has been advocated by some authors. Given recent evidence that such increases in fluid can be dangerous in marathon runners, another group that sometimes uses this practice as a preventative measure (Almond *et al*, 2005), advice on fluid intake among MDMA users may warrant reconsideration.

There are a number of caveats that must be considered when interpreting these data. The number and times of collection of blood samples was limited and it is possible that many of the subjects had higher drug concentrations at times when samples were not taken. More frequent blood sampling and measurements of drug concentrations and effects are required to clarify the pharmacokinetic/pharmacodynamic relationships for these drugs, and these studies are planned. In addition, the role of 'other drug use' on the pharmacokinetic and pharmacodynamics of MDMA is not clear, and could have influenced study results, along with numerous other uncontrolled variables, such as activity, temperature, and fluid intake, to name a few. Nevertheless, given that these variables are not controlled in the 'real life setting' of MDMA use, the data are likely to be clinically relevant.

In conclusion, the present results indicate that some individuals who use MDMA in a recreational setting develop plasma concentrations of MDMA that overlap with those seen in non-human primates that develop persistent deficits of brain serotonin axonal markers (Mechan *et al*, 2006, same issue). Notably, these plasma concentrations fall into the 'toxic to lethal' range, according to forensic laboratory standards, yet subjects in the present study had no clinical complaints and, for the most part, had unremarkable vital signs, hematologic and clinical chemistry profiles. Although subjects with the highest plasma concentrations of MDMA tended to have elevated temperatures hours after drug ingestion, temperatures were not clinically worrisome. Additional studies are required to better characterize the pharmacokinetic and pharmacodynamic profiles of MDMA when used in a naturalistic setting, and to better understand the potential influences of 'other drug use', temperature, and activity on these same profiles.

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