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Effect of 3,4-methylenedioxymethamphetamine ("ecstasy") on body temperature and liver antioxidant status in mice: influence of ambient temperature

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Abstract The consumption of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) is known to cause severe hyperthermia and liver damage in humans. The thermogenic response induced by MDMA is complex and partially determined by the prevailing ambient temperature (AT). This is of extreme importance since ecstasy is often consumed at "rave" parties, where dancing takes place in a warm environment, which may exacerbate the effect of MDMA on thermoregulation. In view of the fact that hyperthermia is a well-known prooxidant aggressive condition, its potential role in ecstasy-induced hepatocellular toxicity should be further studied. Thus, the present study was performed in order to evaluate the influence of AT on the effects of single administration of MDMA on body temperature and liver toxicity in Charles River mice. Animals were given an acute intraperitoneal dose of MDMA (5, 10 or 20 mg/kg) and placed in AT of 20 ± 2°C or 30 ± 2°C for 24 h. Body temperature was measured during the study using implanted transponders and a temperature probe reading device. Plasma and liver samples were used for biochemical analysis. Liver sections were also taken for histological examination. The parameters evaluated were (1) plasma levels of transaminases and alkaline phosphatase, (2) hepatic glutathione (GSH), (3) hepatic lipid peroxidation, (4) activity of hepatic antioxidant enzymes (catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase, copper/zinc superoxide dismutase and manganese superoxide dismutase), and (5) liver histology. The hyperthermic response elicited by MDMA was clearly dose-related and potentiated by high AT. Administration of MDMA produced some evidence of oxidative stress, expressed as GSH depletion at both ATs studied, as well as by lipid peroxidation and decreased catalase activity at high AT. High AT, by itself, decreased glutathione peroxidase activity. Histological examination of the liver revealed abnormalities of a dose- and AT-dependent nature. These changes included vacuolation of the hepatocytes, presence of blood clots and loss of typical hepatic cord organisation. The results obtained in the present study suggest that oxidative stress plays a part in the first stage of MDMA-induced liver damage and that liver antioxidant status is aggravated by increased AT. Thus, these findings are in accordance with the hypothesis that high AT may potentiate ecstasy-induced hepatotoxicity by increasing body hyperthermia.

Keywords 3,4-Methylenedioxymethamphetamine (MDMA) · Ambient temperature · Hyperthermia · Hepatotoxicity · Oxidative stress · Mice

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Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy), a ring-substituted amphetamine derivative, has attracted a great deal of media attention in recent years due to its widespread abuse as a recreational drug by the young generation. Clinical evidence has shown that the liver is a target organ for MDMA toxicity; in this sense, histological examinations in some studies have shown several changes ranging from a mild lobular hepatitis to submassive lobular collapse (Ellis et al. 1996; Milroy et al. 1996; Fineschi et al. 1999). In addition, a case of accelerated hepatic fibrosis in association with ecstasy use has been reported (Khakoo et al. 1995). Although there are several reports of liver damage after ecstasy consumption by humans, the respective underlying mechanisms are still under investigation. Various factors probably play a role in ecstasy-induced hepatotoxicity, namely its metabolism, the increased efflux of neurotransmitters, the oxidation of biogenic amines, and

hyperthermia. Ecstasy users may be particularly predisposed to hyperthermia by the direct effects of this drug on the thermoregulatory system. In fact, many patients who have developed hepatocellular damage after ingesting ecstasy have been hyperpyrexial for several hours (Henry et al. 1992; Burnat et al. 1996). It must be emphasized that hyperthermia is a well known pro-oxidant aggressive condition, which leads to irreversible hepatocellular injury both in vitro (Skibba et al. 1991; Carvalho et al. 1997) and in vivo (Ando et al. 1994). The synergistic toxicity of MDMA and hyperthermia in isolated mouse hepatocytes has been reported recently (Carvalho et al. 2001). Misuse of MDMA in crowded conditions with high ambient temperature (AT), physical activity, and dehydration, i.e., under the conditions that MDMA is used at "rave" parties, may all contribute to exacerbate the hyperthermic response of MDMA (Green et al. 1995). It has previously been shown that administration of MDMA to rats produce hyperthermia and this response is sensitive to the prevailing AT (Gordon et al. 1991; Malberg and Seiden 1998). In view of this, the present work was performed to evaluate the influence of AT on the effects of MDMA on mice body temperature and liver toxicity.

Materials and methods

Chemicals

All reagents were of analytical grade. Water for high-performance liquid chromatography (HPLC) was deionised (≤ 0.055 µs/cm; Seral Water Purification Systems, Munich, Germany). Glutathione (GSH), 2-thiobarbituric acid, and all the reagents for enzymatic determinations were obtained from Sigma Chemical Company (St. Louis, Mo., USA). All other chemicals were obtained from Merck (Darmstadt, Germany). MDMA (HCl salt) was generously supplied by the United Nations Drug Control Program (Vienna, Austria).

Animals

Adult male Charles River mice (Instituto Gulbenkian de Ciências, Portugal), weighing 25–30 g, were used in all experiments. For at least 2 weeks prior to use, the mice were housed in groups of five in polyethylene cages at a room temperature of 20 ± 2°C, relative humidity of 50% and under 12 h light/dark cycle (light on 8.00 to 20.00 hours), in our animal house. Standard food and water were available ad libitum except on the drug-injection day, when animals were deprived of food. Housing and experimental treatment of the animals were in accordance with National Institutes of Health guidelines (ILAR 1996). The experiments complied with the current laws of Portugal.

Drug administration

MDMA was dissolved in 0.9% NaCl (saline) and injected intraperitoneally at 5, 10 or 20 mg/kg in a volume of 0.1 ml/100 g body weight. Control animals were injected with saline solution.

Experimental procedure

At 9.00 hours mice were injected with saline or MDMA (5, 10 and 20 mg/kg i.p.) and immediately placed inside the environmental chamber for 24 h. The experiment was performed using two ATs: 20°C and 30°C (± 2°C). Subcutaneous temperature of animals was measured during the experiments using a novel temperature reading device (see Measurement of body temperature). After 24 h the animals were anaesthetized with diethyl ether, blood was removed from the inferior vena cava and collected into heparinized tubes. Plasma and liver samples were used for biochemical analysis. Liver sections were also taken for histological examination.

Measurement of body temperature

Subcutaneous temperatures of the mice were taken using BioMedic programmable temperature transponders (microchips BMDS IPTT-100, Plexx BV, 6660 AE Elst, Netherlands). The microchips were implanted subcutaneously between the shoulder blades using the BioMedic implant device one day before the experiment. Readings of subcutaneous temperature were taken immediately prior to dosing and for 12 h thereafter using the hand-held Biomedic Pocket Scanner (DAS-5007) held approximately 2 cm away from the animal. By this means, temperature was recorded every 15 min during the first hour, every 30 min during second and third hours and every hour thereafter.

It is noteworthy that body temperature is usually measured as rectal temperature with a rigid steel probe thermometer. The invasive nature of this procedure creates some limitations. The stress associated with handling and restraint may itself increase baseline body temperature (Cabanac and Briese 1992; Cilia et al. 1998). On the other hand, repeated sampling increases the risk of rectal bruising and tearing. To overcome this problem, we used a novel temperature measurement apparatus that has as advantages the elimination of hyperthermic effects induced by the stress of restraint and the ability to record responses as often as required.

Biochemical analysis

Plasma levels of transaminases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and alkaline phosphatase (ALP) were determined using appropriate kits (Boehringer Mannheim GmbH Diagnostica, Germany) with an automatic analyser (Cobas Mira S).

GSH measurement was performed by HPLC with a dual-electrode coulometric detection (Remião et al. 2000). Briefly, liver samples were homogenized in 5% perchloric acid with an Ultra-Turrax device. The homogenates were centrifuged at 6,000 *g* for 10 min at 4°C, and 20 µl of each supernatant was then injected into a HPLC system with electrochemical detection.

The extent of lipid peroxidation was measured by the assay for thiobarbituric acid reactive substances (TBARS) at 535 nm. TBARS were expressed as malondialdehyde equivalents, calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Buege and Aust 1978).

For measurement of the activities of the antioxidant enzymes, liver samples were homogenized in 0.1% Triton X-100 in 50 mM phosphate buffer (pH 7.4). The homogenates were centrifuged at 13,000 rpm for 5 min at 4°C, and the supernatants collected for the enzymatic and protein assays. Protein was determined by the Lowry method (Lowry et al. 1951) using bovine serum albumin as standard.

Catalase activity was measured according to the method of Aebi (1984) by monitoring the decomposition of H_2O_2 at 240 nm (extinction coefficient 0.00394 ± 0.0002 mM^{-1} mm^{-1}). The enzyme activity was expressed as micromoles of H_2O_2 consumed/min per mg protein. Glutathione reductase (GRed) activity was assayed spectrophotometrically by following nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm (extinction coefficient 6.2 mM^{-1} cm^{-1}) (Carlberg and Mannervik 1985). The enzyme activity was expressed as micromoles of NADPH per minute per milligram of protein. Glutathione-*S*-transferase (GST) was assayed according to the method of Habig et al. (1974). The formation of GSH conjugate with 1,2-chloro-2-dinitrobenzene was monitored for 5 min at 340 nm. The GST activity was calculated using an extinction coefficient of 9.6 mM^{-1} cm^{-1} and expressed as

micromoles per minute per milligram of protein. Selenium-dependent glutathione peroxidase (GPx) activity was assayed by NADPH oxidation at 340 nm when oxidized glutathione (GSSG) is reduced back by glutathione reductase (Flohé and Gunzler 1984). The enzyme activity was expressed as nanomoles of NADPH/min per mg protein. Copper/zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD) were assayed using the method of Flohé and Ötting (1984). A xanthine-xanthine oxidase system was used to generate superoxide radicals ($O_2^{\cdot-}$), and subsequent reduction of cytochrome *c* by $O_2^{\cdot-}$ was monitored at 550 nm. Potassium cyanide (2 mM) was used to allow the measurement of MnSOD. Enzyme activity was expressed as Units per milligram of protein (1 Unit of SOD is defined as the amount of enzyme required to inhibit the rate of cytocrome *c* reduction by 50%).

Histological analysis

Immediately after sacrificing the animals, liver sections were excised and fixed in Bouin's solution, dehydrated in an ethanol series and embedded in paraffin for routine histology. Sections 5- to 7-μm thick were then stained with haematoxylin and eosin, and examined for histopathology by light microscopy. Each slide was assessed for particular histological alterations. Presence of blood clots was assessed in each slide and the results allocated to one of four categories: - absence in all slides, + present in less than 25% of the slides, + + present in 25 to 75% of the slides, and + + + present in more than 75% of the slides. Hepatocyte vacuolation was assessed in each slide by counting the number of vacuolised hepatocytes among 100 cells analysed, and the results allocated to similar categories as above. Loss of typical hepatic cords was assessed on centrolobular regions of each slide, and allocated to categories as above.

Data analysis

Results are given as mean ± SEM. For temperature analysis, comparisons were made by analysis of variance (ANOVA) for repeated measures, followed by Duncan's Multiple Range Test. All others statistical analyses were made with one-way ANOVA followed by Fisher's Protected Least Significant Difference (PLSD) test. The level of significance was set at $P < 0.05$.

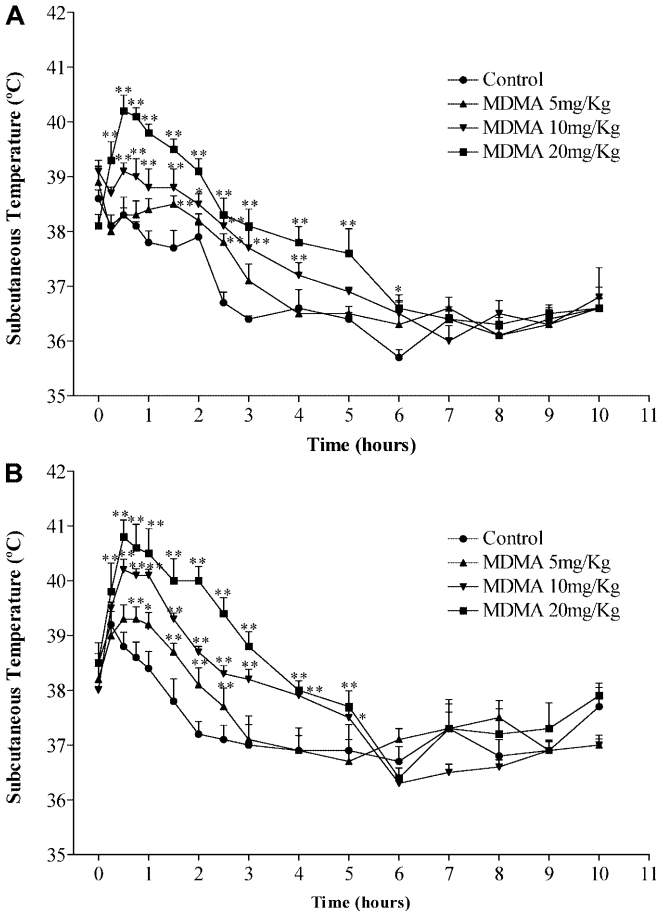

Fig. 1A,B. Effect of 3,4-methylenedioxymethamphetamine (MDMA; 5, 10 and 20 mg/kg i.p.) on subcutaneous temperature when mice were kept at (**A**) normal ambient temperature (20 ± 2°C) and (**B**) high ambient temperature (30 ± 2°C). All results are shown as mean ± SEM of $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, control versus MDMA-treated group

Results

Effect of MDMA on body temperature of mice at normal and high AT

MDMA (5, 10 or 20 mg/kg i.p.) produced a marked increase in mice body temperature, which reached its maximum at approximately 30 min and remained elevated for more than 4 h (Fig. 1A). The hyperthermic response was clearly dose-dependent, with the largest change being approximately 2°C at the highest dose administered. Figure 1B shows the effect of high AT (30 ± 2°C) on MDMA-induced hyperthermia. The highest body temperatures attained in the animals were significantly higher in mice kept at high AT. Maximum temperatures of MDMA-treated mice were 0.6–1.2°C higher at an AT of 30 ± 2°C than at 20 ± 2°C (depending on the administered dose). Overall, the body temperature in control animals was slightly higher at 30 ± 2°C AT than at 20 ± 2°C AT. The statistical comparison indicated a few points of significance, but during the first 2.5 h only one time point was found to be significant (0.25 h, $P < 0.05$). However, MDMA-treated mice had distinct body temperature profiles at the different ATs. Both saline and MDMA-treated mice showed similar body temperatures by 10 h after injection. For this reason, all figures and statistical analysis of body temperature values include only the first 10 h of the experiment.

Lethality

Lethality was only observed for the MDMA dose of 20 mg/kg. Deaths occurred within approximately 3 h post-injection. High AT potentiated the effects of MDMA: 10% lethality was obtained in mice treated with 20 mg/kg MDMA at normal AT and approximately 40% lethality was found for the same dose at high AT. Mice that died during the assay were replaced by others from a satellite group injected with the same dose in order to have always five animals per group since it was previously demonstrated that aggregation of mice affects core temperature (Moore 1963).

Table 1. Effect of 3,4-methylenedioxymethamphetamine (MDMA; 5, 10, 20 mg/kg, i.p.) on liver thiobarbituric acid reactive substances, glutathione and antioxidant enzymes activity 24 h post-dose at normal (20 ± 2°C) and high ambient temperature (30 ± 2°C). The values are reported as mean ± SEM, $n = 10$. (*TBARS* thiobarbituric acid reactive substances as nmol malondialdehyde equivalents/g of tissue; *GSH* glutathione as μmol/g of tissue; *catalase* as μmol H_2O_2/min per mg protein; *GRed* glutathione reductase activity as nmol NADPH/min per mg protein; *GST* glutathione-*S*-transferase as nmol of conjugate/min per mg protein; *GPx* glutathione peroxidase as nmol NADPH/min per mg protein; *MnSOD* and *CuZnSOD* manganese and copper/zinc superoxide dismutase, respectively, both expressed as U/mg protein)

Treatment	Ambient temperature	TBARS	GSH	Catalase	GRed	GST	GPx	MnSOD	CuZnSOD
Saline	20 ± 2°C	15.0 ± 1.2	4.46 ± 0.16	323.7 ± 13.1	23.5 ± 0.9	1237.2 ± 59.0	480.3 ± 14.1	0.78 ± 0.03	12.74 ± 0.69
	30 ± 2°C	15.1 ± 1.2	4.06 ± 0.20	315.4 ± 10.4	22.7 ± 0.5	1153.5 ± 43.4	403.6 ± 17.3$^{\#\#}$	0.79 ± 0.03.	14.37 ± 0.70
MDMA	20 ± 2°C	13.6 ± 0.6	3.91 ± 0.18**	306.5 ± 12.9	23.7 ± 0.7	1380.9 ± 86.4	493.5 ± 17.0	0.87 ± 0.06	16.22 ± 0.73
5 mg/kg	30 ± 2°C	17.1 ± 1.5	3.99 ± 0.13	297.1 ± 13.7	22.3 ± 0.7	1129.4 ± 34.1	394.7 ± 17.9$^{\#\#}$	0.79 ± 0.03	11.16 ± 0.60
MDMA	20 ± 2°C	14.9 ± 1.1	3.49 ± 0.09**	312.7 ± 11.0	23.0 ± 1.0	1301.9 ± 68.6	466.6 ± 14.3	0.85 ± 0.02	11.79 ± 0.61
10 mg/kg	30 ± 2°C	18.1 ± 1.7	3.61 ± 0.21	250.5 ± 10.7**	22.2 ± 0.8	1051.4 ± 49.1	380.8 ± 15.5$^{\#\#}$	0.83 ± 0.03	13.85 ± 0.71
MDMA	20 ± 2°C	11.8 ± 1.0	3.91 ± 0.09**	304.4 ± 19.1	24.5 ± 1.1	1320.8 ± 69.1	466.2 ± 11.5	0.85 ± 0.03	14.52 ± 0.90
20 mg/kg	30 ± 2°C	19.8 ± 1.8*	3.45 ± 0.13**	269.9 ± 10.1*	24.0 ± 0.8	1079.5 ± 44.5	403.9 ± 11.9$^{\#\#}$	0.83 ± 0.03	12.53 ± 0.44

*$P < 0.05$, **$P < 0.01$, for control versus MDMA-treated group
$^{\#\#}P < 0.01$, for 20 ± 2°C versus 30 ± 2°C

Effect of MDMA on liver of mice at normal and high AT

Plasma levels of ALT, AST and ALP were found to be similar among the groups under study (data not shown). Table 1 presents the results from liver TBARS, GSH levels and antioxidant enzymes activities (GRed, GST, GPx, catalase, CuZnSOD and MnSOD) 24 h after MDMA administration at normal and high AT. A significant depletion of liver GSH levels was observed in MDMA-treated groups at both ATs. No significant changes in lipid peroxidation were observed at normal AT but a significant increase of TBARS was found for the highest dose (20 mg/kg i.p.) in animals kept at high AT. Concerning the antioxidant enzyme activities, a significant decrease of catalase activity was observed in animals treated with MDMA 10 and 20 mg/kg at high AT. In addition, a significant decrease on GPx activity in saline-treated mice kept at high AT relative to those at normal AT was observed. Liver GRed, GST, CuZnSOD and MnSOD were found to be similar among the groups at both ATs.

Hepatic sections from control animals showed normal morphology (Fig. 2A). Histological examination of the liver from MDMA-treated mice revealed damage throughout the entire hepatic lobule. Modifications on the centrolobular area did not differ from the other zones of the lobular architecture. Vacuolation and degeneration of the hepatic cells, presence of blood clots and disorganisation of the hepatic parenchyma were noted, with loss of typical hepatic cords radiating from central vein. Increasing dose and AT (see Fig. 2B, C and Table 2) aggravated this feature.

Discussion

Liver damage after MDMA ingestion is an important and often concealed cause of hepatitis or acute hepatic failure, particularly in young people (Henry et al. 1992; Dykhuizen et al. 1995; Ellis et al. 1996; Andreu et al. 1998; Riordan and Williams 1998). The aetiology of the hepatocellular injury is probably multifactorial but hyperthermic liver injury seems to play a part. Hyperthermia is a major clinical feature of MDMA intoxication (Screaton et al. 1992; Dar and McBrien 1996; Walubo and Seger 1999). The thermogenic response to MDMA results from augmentation of brain serotonin levels, which stimulate the thermal control regions in the anterior hypothalamus/pre-optic area. As a result, the temperature set-point increases, which stimulates the sympathetic centre and increases sympathetic discharge (Dafters and Lynch 1998; Walubo and Seger 1999). Catecholamines are released and these stimulate α- or β-adrenergic receptors (depending on the particular tissue's receptor reserve), which increases mitochondrial metabolism and heat generation. Also, muscular contractions and peripheral vasoconstriction contribute to body temperature elevation (Walubo and Seger 1999). However, while the MDMA-induced hyperthermia in experimental animals is dose-related (O'Shea et al. 1998, and present work), in humans this effect is not always related to the amount of drug ingested (Henry et al. 1992; Dar and McBrien 1996). What predisposes certain individuals to develop hyperthermia following MDMA ingestion is not known. Experimentally, it is suggested that a combination of direct effects of the drug and the circumstances associated with ingestion may be involved. MDMA-induced hyperthermia in rats (Gordon et al. 1991) and mice (as shown in the present study) is more pronounced at high AT. In humans, most of the serious and fatal cases encountered were among people at crowded parties or clubs where dancing takes place in a warm environment. At such rave or dance parties, sustained physical activity, a high AT, and inadequate fluid replacement could all reduce heat loss and potentiate a direct effect of the drug on thermoregulatory mechanisms, which may lead to severe or even fulminant hyperthermia. We report that the body temperature in control animals was slightly higher

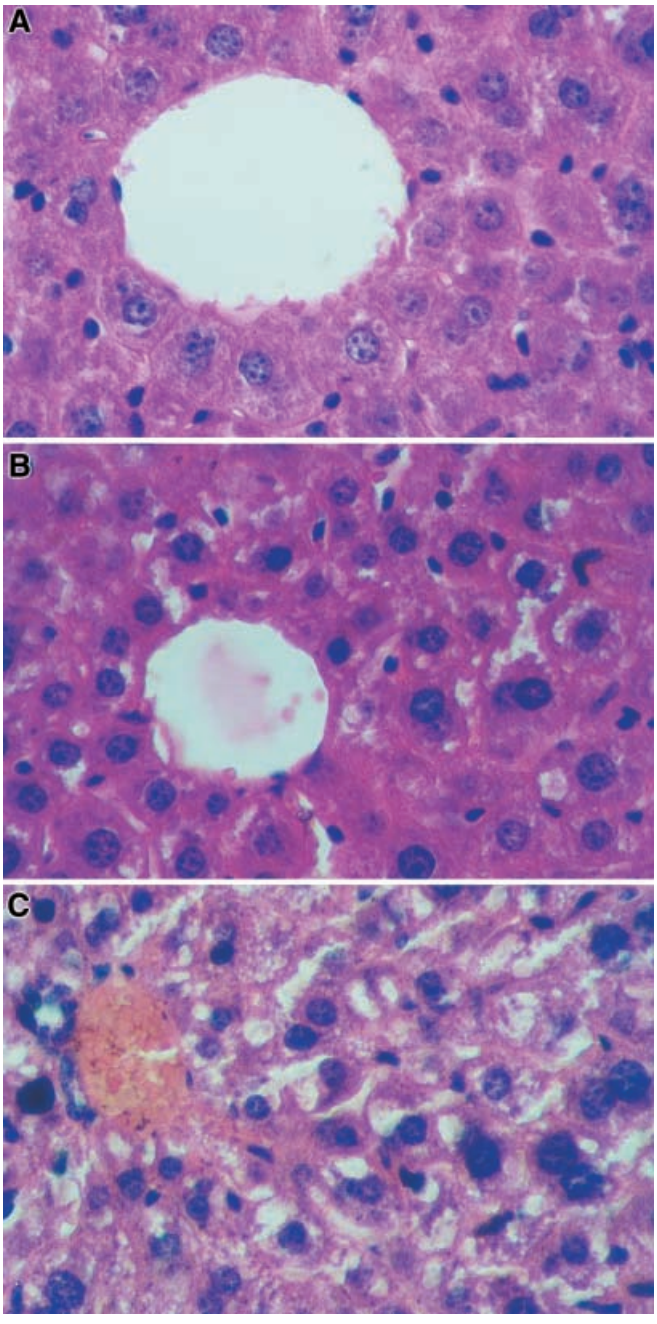

Fig. 2A–C. Liver histopathology of (**A**) saline-treated mice (control group) at high ambient temperature (30 ± 2°C), (**B**) 20 mg/kg MDMA-treated mice at normal ambient temperature (20 ± 2°C), and (**C**) 20 mg/kg MDMA-treated mice at high ambient temperature (30 ± 2°C). Hepatic cells of control group show normal morphology. Liver from MDMA-treated mice reveals vacuolation of the hepatocytes, presence of blood clots and loss of typical hepatic cords organisation. Haematoxylin-eosin stain; original field magnification ×800

at AT of 30 ± 2°C than at 20 ± 2°C AT. However, after administration of MDMA the differences between these ATs were much more evident. This indicates that MDMA may compromise thermoregulatory ability, and this in turn makes the core temperature dependent on the AT. The ambient thermal environment also had a major impact on the extent of MDMA-induced lethality, suggesting a relationship between MDMA-induced hyperthermia and its lethal effects.

It has been shown in vivo that hyperthermia causes lipid peroxidative damage in rat liver (Skibba et al. 1991; Ando et al. 1994) and in freshly isolated rat and mouse hepatocytes (Carvalho et al. 1997, 2001). Oxidative stress has been suggested as the mechanism underlying heat-induced liver toxicity (Skibba et al. 1991). The source of free radicals is probably intracellular because superoxide production by xanthine oxidase has been identified as a source of oxygen-derived free radical formation in the liver during hyperthermia (Skibba et al. 1989a, 1989b). These radicals can be generated after the hyperthermia-induced conversion of xanthine dehydrogenase to the xanthine oxidase form, with the subsequent generation of superoxide (Powers et al. 1992) and hydroxyl radicals (Flanagan et al. 1998), and in the presence of free iron (released from ferritin by superoxide radical) as the catalyst in the Fenton reaction (Powers et al. 1992).

In view of the fact that hyperthermia is a pro-oxidant aggressive condition, its potential role in ecstasy-induced hepatocellular toxicity needs to be further studied. The potentiation by hyperthermia of toxicity elicited by MDMA upon freshly isolated mouse hepatocytes has been reported recently (Carvalho et al. 2001). Hyperthermia potentiated MDMA-induced depletion of GSH, production of lipid peroxidation and loss of cell viability up to 90–100%.

In the present study, several parameters indicative of liver oxidative stress after acute MDMA administration were examined. GSH depletion after MDMA administration was observed at both ATs. There is evidence that MDMA is biotransformed into a reactive metabolite capable of conjugating with glutathione (Hiramatsu et al. 1990). MDMA is demethylenated to α-methyldopamine (α-MeDA) by cytochromes P450 2D, 2B and 3A1 (Kreth et al. 2000). α-MeDA is readily oxidised to the corresponding *o*-quinone, followed by conjugation with glutathione to form 5-(glutathion-*S*-yl)-α-MeDA (Hiramatsu et al. 1990). It is noteworthy that the conjugate can still be converted into a glutathione-*o*-quinone and undergo addition of a second molecule of GSH to form 2,5-bis(glutathion-*S*-yl)-α-MeDA (Monks and Lau 1997). The reactive *o*-quinone intermediates are Michael-acceptors, and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. Alternatively, quinones are highly redox-active molecules which can enter the redox cycle with their semiquinone radicals, leading to formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical (Bolton et al. 2000). The MDMA-induced GSH depletion observed in the present work has already been demonstrated in vivo in rat liver (Beitia et al. 2000) and in vitro in freshly isolated mice hepatocytes (Carvalho et al. 2001). Glutathione is an important cellular antioxidant and plays a major role in protecting cells against oxidative stress (Sciuto 1997).

Table 2. Effect of 3,4-methylenedioxymethamphetamine (MDMA; 5, 10, 20 mg/kg, i.p.) on liver histology 24 h post-dose at normal (20 ± 2°C) and high ambient temperature (30 ± 2°C). Extent of lesions in the hepatic parenchyma: – absence in all slides; + present in less than 25% of the slides; + + present in 25 to 75% of the slides; + + + present in more than 75% of the slides

Treatment	Ambient temperature	Histological findings		
		Presence of blood clots	Hepatocyte vacuolation	Loss of hepatic cords
Saline	20 ± 2°C	–	–	–
	30 ± 2°C	–	–	–
MDMA 5 mg/kg	20 ± 2°C	–	+	–
	30 ± 2°C	–	+	+
MDMA 10 mg/kg	20 ± 2°C	–	+	+
	30 ± 2°C	+	+ +	+
MDMA 20 mg/kg	20 ± 2°C	+	+ +	+
	30 ± 2°C	+ +	+ + +	+ + +

Decreased levels of GSH in the liver may compromise cellular antioxidant defences and render the cells more exposed to the deleterious effects of ROS being formed within the cells. It is noteworthy that the anorectic effect of MDMA will make the depletion of GSH and other protective agents like vitamins E and C in liver even more dramatic. In addition, not only GSH itself but also catalase and GPx contributes to radical scavenging. The data reported in this study shows a significant decrease on catalase activity in animals treated with MDMA at high AT. In addition, high AT, by itself, decreased GPx activity. Catalase, GPx and GSH represent a major pathway in the cell for metabolising hydrogen peroxide. Thus, depletion of GSH and/or decreased activity of these enzymes may compromise this pathway and thereby allow hydrogen peroxide to accumulate to toxic levels. It must be stressed that hepatic mitochondria are especially vulnerable to this effect since they lack the hydrogen peroxide metabolising enzyme catalase (DeLeve and Kaplowitz 1991). Alternatively, the loss of GSH may lead to alterations in normal cellular metabolism and lead to the increased accumulation of ROS.

Lipid peroxidation showed a tendency to increase after MDMA injection at high AT, which was significant ($P < 0.05$) for the highest dose tested. One of the principal biological effects of lipid peroxidation is membrane damage. However, in this study membrane damage, as reflected by leakage of the intracellular AST and ALT, was not observed. These biochemical findings are corroborated with liver histology, which revealed no evidence of hepatic necrosis. Nevertheless, histological examination of the liver from MDMA-treated mice revealed damage throughout the entire hepatic lobule, namely vacuolation of the hepatic cells, presence of blood clots in hepatic veins and loss of typical hepatic cords. These lesions were clearly potentiated by increasing dose and AT. Disseminated intravascular coagulation (DIC) is a frequent clinical feature of ecstasy intoxication (Henry et al. 1992; Walubo and Seger 1999) that results in varying degrees of microvascular obstruction due to fibrin-platelet deposition. We suggest that DIC induced by MDMA probably explains the observed presence of blood in hepatic vein.

Overall, the results obtained in the present study suggest that oxidative stress plays a part in the first stage of MDMA-induced liver damage and that liver antioxidant status is aggravated by increased AT. MDMA induced vacuolation and degeneration of the hepatic cells, the occurrence of blood clots and disorganisation of the hepatic parenchyma. Subsequent attacks by repeated administration of the drug may dramatically aggravate cellular antioxidant defences and result in irreversible hepatocellular injury.

A possible explanation for the liver damage reported for MDMA may be the following: after its administration, MDMA undergoes hepatic metabolic activation, reacts with glutathione and probably other intracellular sulphydryl groups. At the same time, MDMA increases body temperature. The liver, partially depleted of sulphydryl groups, would then be exposed to hyperthermia, which seems to exert damage mainly via oxidative stress. This putative toxicological mechanism is reinforced by the results obtained in the present study.

In summary, these data indicate that ambient temperature has a significant effect on body temperature of MDMA-treated mice. Since most of the serious cases of hyperthermia after ecstasy abuse are encountered in hot environments, this strongly suggests that the effect of MDMA on thermoregulation extend to humans in these circumstances. Furthermore, the present results corroborate the hypothesis that ambient temperature may enhance liver toxicity by increasing body hyperthermia.

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