DMD Fast Forward. Published on July 19, 2007 as doi:10.1124/dmd.107.016261

Ambient Temperature Effects on 3,4-methylenedioxymethamphetamine (MDMA)-induced Thermodysregulation and Pharmacokinetics in Male Monkeys

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Running Title: Ambient temperature and MDMA disposition in monkeys

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Text pages: 14

Tables: 1

Figures: 3

References: 34

Words in Abstract: 250

Words in Introduction: 649

Words in Discussion: 1015

Abbreviations: 3,4-methylenedioxymethamphetamine (MDMA); 3,4-methylenedioxyamphetamine (MDA); 3,4-dihydroxymethamphetamine (HHMA); 3,4-dihydroxyamphetamine (HHA); cytochrome P450 (CYP); maximum plasma concentration (C_{max}); area under the concentration-time curve (AUC); elimination half-life ($t_{1/2}$); time of maximum concentration (T_{max}); volume of distribution at steady-state (Vd_{ss}); vascular access port (VAP); solid-phase extraction (SPE); strong cation exchange (SCX); liquid chromatography/tandem mass spectrometry (LC-MS/MS)

ABSTRACT:

Changes in ambient temperature are known to alter both the hyperthermic and the serotonergic consequences of 3,4-methylenedioxymethamphetamine (MDMA). Metabolism of MDMA has been suggested to be a requisite for these neurotoxic effects, while the hyperthermic response is an important contributing variable. The aim of the present study was to investigate the interaction between ambient temperature, MDMA-induced thermodysregulation, and its metabolic disposition in monkeys. MDMA (1.5 mg/kg, IV) was administered noncontingently at cool (18°C; n=5), room (24°C; n=7) and warm (31°C; n=7) ambient temperatures. For 240 min following MDMA administration, core temperature was recorded and blood samples were collected for analysis of MDMA and its metabolites 3,4-dihydroxymethamphetamine (HHMA), 3,4-dihydroxyamphetamine (HHA), and 3,4-methylenedioxyamphetamine (MDA). A dose of 1.5 mg/kg MDMA induced a hypothermic response at 18°C, a hyperthermic response at 31°C, and did not significantly change core temperature at 24°C. Regardless of ambient temperature, plasma MDMA concentrations reached maximum within 5 min, and HHMA was a major metabolite. Curiously, the approximate elimination half-life ($t_{1/2}$) of MDMA at 18°C (136 min) and 31°C (144 min) was increased compared to 24°C (90 min) and is most likely due to volume of distribution changes induced by core temperature alterations. At 18°C, there was a significantly higher MDA area under the concentration-time curve (AUC) and a trend for a lower HHMA AUC compared to 24°C and 31°C, suggesting that MDMA disposition was altered. Overall, induction of hypothermia in a cool environment by MDMA may alter its disposition. These results could have implications for MDMA-induced serotonergic consequences.

The recreational drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is often used at “raves” or dance parties (Irvine et al., 2006), which are characterized by marathon, vigorous group dancing with laser light displays, hypnotic electronic music, hot environmental conditions, and concurrent use of other illicit drugs (Schwartz and Miller, 1997; Weir, 2000). In this setting, ecstasy can induce a hyperthermic response that is life-threatening (Mallick and Bodenham, 1997). Ambient temperature, which is often elevated at these dance parties, is known to alter the thermogenic (Gordon et al., 1991; Von Huben et al., 2007), neurochemical (O’Shea et al., 2005), behavioral (Cornish et al., 2003), and neurotoxic effects of MDMA (Malberg and Seiden, 1998). However, the interaction of ambient temperature and MDMA pharmacokinetics has not been investigated.

MDMA thermodysregulation in laboratory animals is dependent upon the ambient temperature. That is, a hyperthermic response occurs at ambient temperatures greater than 24°C and a hypothermic response occurs at ambient temperatures of 20°C or lower (Gordon et al., 1991; Taffe et al., 2005; Von Huben et al., 2007). Although a hyperthermic response to MDMA is not essential for subsequent serotonergic neurotoxicity (Shankaran et al., 1999; Falk et al., 2002; Darvesh et al., 2004; Jones et al., 2005), experimental manipulations, such as pharmacological agents (Farfel and Seiden, 1995; Malberg et al., 1996), surgical removal of pituitary and thyroid glands (Sprague et al., 2003), and changes in ambient temperature (Malberg and Seiden, 1998), that attenuate MDMA-induced hyperthermia also attenuate the subsequent neurotoxic effects. A better understanding of MDMA disposition in nonhuman primates at cool, room, and warm ambient temperatures may provide insight into any relationship between ambient temperature, thermoregulation, MDMA metabolism, and subsequent neurotoxicity.

MDMA disposition has been assessed previously in rodents, nonhuman primates, and humans (see de la Torre et al., 2004; Monks et al., 2004; de la Torre and Marré, 2005). Briefly, the major metabolic pathway in humans involves *O*-demethylenation to 3,4-dihydroxymethamphetamine (HHMA) by the cytochrome P450 isozyme 2D6 (CYP2D6; Tucker et al., 1994; Kreth et al., 2000; Fig. 1). A minor pathway in humans involves *N*-demethylation to 3,4-methylenedioxyamphetamine (MDA) by CYP2B6, which can be further *O*-demethylenated to 3,4-dihydroxyamphetamine (HHA; Mauerer et al., 2000; Segura et al., 2001; Fig. 1). HHMA also can be *N*-demethylated to HHA by CYP2B6. HHMA and HHA have been postulated by some to be the neurotoxic metabolites of MDMA (de la Torre and Marré, 2004; Jones et al., 2005). Species differences in MDMA metabolism are apparent between rodents and humans. For instance, the major pathway of MDMA metabolism in rodents is *N*-demethylation to MDA compared to *O*-demethylenation to HHMA in humans (Lim and Foltz, 1988; de la Torre et al., 2004). In contrast, little is known about MDMA metabolism in nonhuman primates. To date, studies in nonhuman primates have assessed only plasma MDMA and MDA concentrations (Bowyer et al., 2003; Mechan et al., 2005). Thus, there is a critical gap in our knowledge of the metabolic disposition of MDMA in nonhuman primates, regardless of ambient temperature.

The aims of the present study were to characterize MDMA disposition in nonhuman primates, to examine the relationship between the thermodysregulatory effects of MDMA (1.5 mg/kg, IV) and three ambient temperatures (18°C, 24°C, and 31°C) and measure plasma concentrations of MDMA and its metabolites (HHMA, MDA, or HHA). We hypothesized that MDMA would induce a hypothermic response at 18°C; whereas at 24°C, a hyperthermic response would be observed that was potentiated at 31°C. We also hypothesized that MDMA metabolism would be similar to humans and that HHMA would be the primary metabolite. One

potential mechanism by which lowering the ambient temperature protects, and elevating the ambient temperature potentiates, the serotonergic neurochemical deficits of MDMA could be changes in metabolism and plasma concentrations of the putative neurotoxic metabolites HHMA and HHA. We hypothesized that lowering the ambient temperature would decrease HHMA and HHA plasma concentrations, whereas elevating the ambient temperature would increase HHMA and HHA plasma concentrations.

METHODS:

Subjects*:* Adult male rhesus monkeys (*Macaca mulatta*; n=10) served as subjects. Four monkeys were drug-naïve and six monkeys had previous histories of cocaine self-administration (Martelle et al., 2007), but had not self-administered cocaine or any other psychostimulant for at least three months prior to the start of the study. Four subjects were randomly selected and first exposed to MDMA at room temperature, and three subjects were first exposed to MDMA at the warm ambient temperature. After both room and warm ambient temperature studies, five subjects (including some of the earlier subjects) were exposed to MDMA at the cool ambient temperature. Approximately 21 days separated MDMA administrations. Each monkey was surgically prepared with an indwelling intravenous catheter into the femoral vein and a subcutaneous vascular access port (VAP, Access Technologies, Skokie, IL) as previously described (Martelle et al., 2007). All subjects were trained to sit calmly in a primate restraint chair (Primate Products, Redwood City, CA) during the monitoring period. Monkeys were weighed weekly and fed sufficient food daily (LabDiet High Protein Primate Chow #5045 and fresh fruit) to maintain 95% of free-feeding weights; water was available *ad libitum* in the home cage, where monkeys had visual and auditory contact with each other. The facilities for housing

and care of the animals are accredited by the American Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures were approved by the Animal Care and Use Committee (ACUC) of Wake Forest University and performed in accordance with established practices as described in the *National Institutes of Health Guide for Care and Use of Laboratory Animals*, except the elevation of ambient temperature that was outside the *Guide* recommended range. During these experiments, no monkey had a core temperature above 41°C that required intervention or termination of the experiment. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Nonhuman Primate Environmental Enrichment Plan.

Ambient temperature manipulation: While in the restraint chair, subjects were placed in a ventilated and sound-attenuating behavioral chamber (150 x 74 x 76 cm, Med Associates, East Fairfield, VT). An indoor/outdoor thermometer (Oregon Scientific, Tualatin, OR) was threaded through a hole in the chamber and attached to the chamber wall. The digital readout was placed on top of the chamber to allow for continuous recording and adjustments to maintain the ambient temperature within ± 1°C of target temperature without opening the chamber door. To elevate the ambient temperature, a commercially available space heater was placed outside one of the vent holes and warm air forced into the chamber. To lower the ambient temperature, a commercially available air conditioner was placed outside the vent holes and cool air forced into the chamber. Monkeys were placed into the chamber and allowed to acclimate to the ambient temperature for approximately 20 min prior to the start of the study.

Temperature Monitoring*:* Rectal temperatures (RET-1 rectal probe attached to a TH-3 thermocouple, Physitemp, Clifton, NJ) were recorded immediately before MDMA administration and each time point corresponding with a blood sample (see below).

Blood sampling*:* Blood samples were taken from the subcutaneous VAP immediately before MDMA administration (1.5 mg/kg, IV) and at 5, 10, 15, 30, 60, 90, 120, 180, and 240 min post-MDMA administration. After MDMA, the VAP was flushed with 5 mL of sterile saline. At each time point, approximately 3 mL of blood was withdrawn using a disposable, sterile, 3-mL syringe attached to a Huber needle. The first mL was discarded to prevent dilution from heparinized saline (100U/mL) in the VAP and catheter. Following each blood sample, approximately 2 mL of heparinized saline was flushed to maintain patency. Blood samples were immediately dispensed into chilled 2-mL Vacutainer® tubes containing K2 EDTA 3.6 mg solution (plasma; Becton-Dickinson, Franklin Lakes, NJ, USA) and stored on ice for up to 20 min until centrifugation. Samples were centrifuged at 3000 rpm for 5-10 min at 4°C; plasma was withdrawn using a disposable pipette and decanted into labeled 1.5-mL microcentrifuge tubes. Next, 250 mM sodium metabisulfite was added (30 μL) to the plasma sample as described in Segura et al. (2001) to minimize oxidation of MDMA and its metabolites. Samples were immediately vortexed and stored at -30°C until analysis.

Materials and Reagents: Racemic MDMA (HCl and base) and MDA base were provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (Research Triangle Park, NC, USA). MDMA HCl was dissolved in 0.9% saline at a concentration of 50 mg/mL for thermoregulation and metabolism studies. MDMA and MDA were dissolved in methanol for

stock and working solutions for analysis. HHMA and HHA were synthesized as previously described (Ensslin et al., 1996; Pizarro et al., 2001) and prepared in methanol for stock and working solutions. Sodium metabisulfite (Sigma, St. Louis, MO, USA) was dissolved in sterile water.

Analytical procedure*:* MDMA, HHMA, HHA and MDA concentrations were analyzed using a modification of previously described methods (Segura et al., 2001; 2002) using acid hydrolysis, solid-phase extraction (SPE) using strong cation exchange (SCX) SPE columns, and liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis.

Calibration, working and control solutions:

For each analytical run, calibration standards ranging from 1 ng/mL to 1000 ng/mL were prepared in clean, separate, silanized 16 x 100 mm glass culture tubes. The appropriate amount of standard working solution was added to 1 mL of 4-fold diluted blank rhesus monkey plasma (0.25 mL monkey plasma plus 0.75 mL Milli-Q water) that also contained 50 µL of 250 mM ascorbic acid, 50 µL of 250 mM sodium EDTA, 50 µL of 250 mM sodium bisulfite, and 50 µL of 4-methyl catechol. The control working solutions were used to prepare quality control samples at 35, 100 and 650 ng/mL.

Sample Preparation:

Plasma samples were analyzed as 4-fold dilutions (0.25 mL of sample plus 0.75 mL of Milli-Q water), 10-fold dilutions (0.10 mL of sample plus 0.90 mL of Milli-Q water) or 50-fold dilutions (0.02 mL of sample plus 0.98 mL of Milli-Q water). The samples were transferred to separate silanized glass culture tubes that contained 50 µL of 250 mM of ascorbic acid, 50 µL of

250 mM of sodium EDTA, 50 µL of 250 mM of sodium bisulfite and 50 µL of 4-methyl catechol.

Extraction preparation:

For internal standard addition, 50 µL containing 1 ng/µL MDMA-d5 and MDA-d5 was transferred to each plasma sample tube. The tubes were then vortexed. For hydrolysis, a 1 mL volume of 0.5 N hydrochloric acid was added to each tube. Teflon-lined screw caps were placed on each tube and the tubes were vortexed. The tubes were then placed in a 100°C oven and allowed to incubate for 30 min. The tubes were then removed from the oven and allowed to cool to room temperature. 100 µL of 70% perchloric acid was added to each tube to precipitate proteins. The tubes were immediately vortexed and then centrifuged for 10 min at 2000 rpm. The supernatants were collected into clean silanized 16 x 100 mm culture tubes. The pH of the sample preparation supernatants was adjusted to pH 6 (as assessed using pH paper) by adding 1 mL of 1 M potassium phosphate, followed by 5 drops of 10 N sodium hydroxide. The tubes were centrifuged as described above and reserved for the SPE.

Solid-phase extraction:

Bond Elute LRC SCX (Varian) SPE columns (1 mL, 100 mg) were used for the extraction procedure. A separate SPE column was used for each submitted sample, calibration standard, or quality control sample. The SPE columns were conditioned by successive addition of 2 mL of methanol, 2 mL of Mill-Q water, and 2 mL of 1 M potassium phosphate. Vacuum was not used in the conditioning steps. The sample preparation supernatants were added to their respective conditioned SPE columns. After addition of the samples, the columns were washed by successive additions of 1 mL of Mill-Q water and 4 mL of methanol. After the methanol passed through the SPE columns, vacuum was applied (> 10 in Hg) for 2 min to dry the columns. After

drying, the SPE columns were eluted by adding 2 mL of methanol:12 M hydrochloric acid (99:1). The eluted samples were collected into clean, separate 5-mL conical glass tubes. A Turbovap was used to evaporate the extracts under a stream of nitrogen in 40°C water. Following evaporation, 200 µL of methanol was added to reconstitute the extracts. To remove particulate material in the extracts, the conical tubes were centrifuged for 10 min at 2000 rpm. The supernatants were transferred to clean separate 5-mL conical glass tubes. The cleaned extracts were evaporated as described above and then reconstituted with 100 µL of 0.1% formic acid:methanol (97:3). The extracts were transferred to clean separate plastic 11-mm conical autosampler vials.

LC-MS analysis:

A Thermo-Finnigan TSQ 7000 MS-MS coupled with an Agilent 1100 HPLC system was used for the analysis. Chromatographic separation utilized a Metasil Basic, 100 x 3.0 mm, 3µ HPLC column (Varian). The mobile phase consisted of a 0.1% formic acid and methanol gradient that varied from 97:3 to 88:12 at a flow rate of 0.2 mL/min. The analytes were ionized by electrospray ionization. The capillary column temperature was set at 250°C. Selected-reaction monitoring was employed. The following transitions were monitored: MDMA (m/z 194→ 163); MDMA-d_5 (m/z 199 →165); MDA (m/z 180→ 163); MDA-d_5 (m/z 185→ 168); HHMA (m/z 182→ 151); and HHA (m/z 168→ 151). MDA-d_5 was also used as the internal standard for HHMA and HHA.

Statistical Analysis: Core temperature data were analyzed with repeated measures mixed linear regression using SAS Proc Mixed (Version 8.2, SAS, Cary, NC) with ambient temperature and time as factors. Tests of the main effects of ambient temperature and time and their interaction

were performed using F-tests. In the presence of a significant interaction, post-hoc least squares means difference tests were performed for planned comparisons between: (1) core temperature at time 0 min and core temperature at each time point after MDMA within an ambient temperature, and (2) each ambient temperature within time 0.

Maximum concentration (C_{max}), time of maximum concentration (T_{max}), volume of distribution at steady-state (Vd_{ss}), clearance (CL), area under the concentration-time curve (AUC), and elimination half-life ($t_{1/2}$) were determined using a non-compartmental IV-bolus model (WinNonlin Professional 5.1, Pharsight Co., Mountain View, CA). Pharmacokinetic data were analyzed with repeated measures mixed linear regression. Ambient temperature was the main factor and a significant main effect was followed by a post-hoc least squares means difference test. All results were considered significant at the 95% level of confidence ($p < 0.05$).

RESULTS:

Effects of ambient temperature on MDMA-induced hyperthermia: Saline administration at the cool, room and warm ambient temperatures did not significantly change core temperature over the 240-min monitoring period (data not shown). When 1.5 mg/kg MDMA was administered intravenously, a significant ambient temperature and time interaction was detected ($F_{14,70}$=7.44, p<0.05). Post-hoc analysis revealed that when administered at room temperature (24°C), MDMA did not significantly change core temperature over the 240 min sampling period. In contrast, at 18°C, this dose of MDMA induced a significant decrease (p<0.05) in core temperature starting at 60 min and lasting through 240 min (Fig. 2). At 31°C, MDMA induced a significant increase (p<0.05) in core body temperature starting at 30 min and lasted through 240 min. Between

ambient temperatures, there was a significant difference ($p<0.05$) only between baseline temperatures at 31°C and 24°C.

Effects of ambient temperature on MDMA disposition: At room temperature (24°C) MDMA C_{max} reached T_{max} at 5 min and was not significantly different when tested at different ambient temperatures (Fig. 3, Table 1). HHMA C_{max} reached T_{max} between 60 to 120 min after MDMA administration regardless of ambient temperature. HHA C_{max} reached T_{max} at ~90 min regardless of ambient temperature. MDA C_{max} also was not significantly affected by ambient temperature. In contrast to the C_{max} data, there was a significant main effect of ambient temperature on MDA $AUC_{0\text{-}240min}$ ($F_{2,3}=11.1$, $p<0.05$). Post-hoc analysis revealed that the AUC at 18°C was significantly greater ($p<0.05$) than either 24°C or 31°C (Table 1).

DISCUSSION:

The aim of the present study was to examine the relationships between ambient temperature, MDMA-induced changes in core temperature and its metabolism. MDMA (1.5 mg/kg, IV) did not affect core temperature when administered at room temperature (24°C), but induced a significant hypothermic response at 18°C and a significant hyperthermic response at 31°C. However, the metabolic disposition of MDMA could not account for the effects of ambient temperature on thermoregulation. Similar to humans, a major route of MDMA metabolism in nonhuman primates involves *O*-demethylenation to HHMA. After HHMA, HHA had the next highest metabolite:MDMA ratio, followed by MDA. At 24°C, the $t_{1/2}$ of MDMA was approximately 90 min. Both increasing and decreasing the ambient temperature significantly increased this approximate $t_{1/2}$. Also, lowering the ambient temperature altered MDMA

disposition such that a significantly greater MDA AUC was observed, with a trend for a decreased HHMA AUC compared to both 24°C and 31°C. These findings indicate a more complex interaction between MDMA and central nervous system function than simply ambient temperature induced changes in metabolism.

Previous nonhuman primate MDMA pharmacokinetic studies (Bowyer et al., 2003; Mechan et al., 2006) have reported only MDMA and MDA plasma concentrations. Thus, data are lacking in nonhuman primates on plasma concentrations of HHMA, which is a major metabolite in humans. Limited *in vitro* evidence (Bogaards et al., 2000) suggests that the rats and monkeys have cytochrome P450 activity similar to humans for the specific isozymes involved in MDMA metabolism. In contrast to the *in vitro* findings, however, *in vivo* studies suggest that species differences exist. MDA is a major metabolite in rats (Chu et al., 1996), whereas HHMA is a major metabolite in monkeys (present study) and humans (Segura et al., 2001; 2002). The results of the present study confirm that MDMA metabolism in monkeys is similar to humans, providing additional support for the use of monkeys to model human MDMA abuse.

A direct comparison between the present study and previous pharmacokinetic studies in monkeys (Bowyer et al., 2003; Mechan et al., 2006) is complicated by the use of different doses and routes of administration. Nevertheless, several general comparisons can be drawn. Although a lower dose was used, the MDMA C_{max} in the present study is at least 5-fold higher than reported by Mechan et al. (2006) or Bowyer et al. (2003). Furthermore, MDMA $t_{1/2}$ (~1.5 hrs. at 24°C) in the present study is decreased compared to 2.8 hrs and ~3.5 hrs. in the Bowyer et al. (2003) and Mechan et al. (2006) studies, respectively. These differences in C_{max} and $t_{1/2}$ can be explained in part by differences in routes of administration. Future studies in nonhuman primates

should extend these characterizations of ambient temperature to oral MDMA administration, which is the primary route used by humans.

Previous research in rodents (Gordon et al., 1991) and monkeys (Von Huben et al., 2007) has demonstrated that ambient temperature can alter the thermogenic effects of MDMA, with MDMA inducing a hypothermic effect at ambient temperatures lower than 20°C and a hyperthermic effect at ambient temperatures greater than 24°C. In contrast to Von Huben et al. (2007), in the present study, MDMA did not significantly change core temperature at 24°C. One potential reason for this discrepancy could be monkeys were seated in restraint chairs in the present study compared to freely moving in the Von Huben et al. (2007) study. However, MDMA-induced hyperthermia in monkeys (and rodents for that matter) does not appear to be related to motor activity (Taffe et al., 2005; Crean et al., 2006; Von Huben et al., 2007). A more likely explanation is that core body temperature was measured in the present study, while (Von Huben et al. (2007) measured temperature using subcutaneous telemetry. This conclusion implies that at least two different processes are operating following MDMA administration that influence thermoregulation. Rodent models have demonstrated that the hyperthermia induced by MDMA results from the activation of the sympathetic nervous system and the hypothalamic-pituitary-thyroid/adrenal axis. The release of norepinephrine mediated by MDMA generates heat through regulation of uncoupling proteins by α_1- and β_3- adrenoreceptors and loss of heat dissipation through norepinephrine-mediated vasoconstriction (for a review see Mills et al., 2004).

Curiously, the $t_{1/2}$ of MDMA was increased both by lowering and elevating the ambient temperature. These data should be considered as approximate estimations of $t_{1/2}$ since the 240-min. sampling period was probably too short to reliably estimate the $t_{1/2}$. Although both CL and

Vd_{ss} are factors affecting $t_{1/2}$, there was no statistically significant difference in CL or Vd_{ss} at the different ambient temperatures. There was a trend, however, for a smaller Vd_{ss} at 18°C, which could increase $t_{1/2}$. Consistent with this possibility, Bansinath et al. (1988) examined the effects of hypo- and hyperthermia on morphine disposition and found increases in $t_{1/2}$ and decreases in Vd_{ss} in hypothermic but not hyperthermic dogs compared to normothermic controls. Another potential reason for the increased $t_{1/2}$ at 18°C could be hypothermia-induced impairment of biliary secretion and microsomal function in the liver (Kalser et al., 1968). MDMA can also induce cutaneous vasoconstriction regardless of ambient temperature (Pedersen and Blessing, 2001), which would presumably decrease the Vd_{ss}. In the present study, however, Vd_{ss} was similar at both 24°C and 31°C, despite different thermogenic responses. Thus, the reasons for similar effects on $t_{1/2}$ of both increasing and decreasing the ambient temperature are unclear, but may involve changes in peripheral circulation.

In conclusion, the results of the present study indicate that a major metabolite of MDMA in nonhuman primates is HHMA, the major MDMA metabolite in humans, confirming that nonhuman primates are a more analogous model than rodents for the pharmacokinetic characterization of MDMA. These results also indicate that either lowering or elevating the ambient temperature increases the $t_{1/2}$ of MDMA. Furthermore, lowering the ambient temperature significantly altered MDMA disposition, shunting metabolism towards the *N*-demethylation pathway and away from the *O*-demethylenation pathway. In contrast, elevating the ambient temperature did not alter the metabolic pathway of MDMA. If changes in ambient temperature and/or core temperature differentially alter MDMA disposition, these results may have implications for the study of MDMA-induced serotonergic neurochemical deficits.

ACKNOWLEDGEMENTS:

We appreciate the statistical assistance of Beth Reboussin and the technical assistance of Rodger Foltz, David Andrenyak, and David Moody at the University of Utah Center for Human Toxicology for analysis of MDMA and its metabolites.

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Footnotes:

This research was supported by National Institute on Drug Abuse grants P50 DA-06634 (MAN) and F31 DA-020281 (MLB). No authors have financial conflicts of interest with the research described in this manuscript.

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DMD Fast Forward. Published on July 19, 2007 as DOI: 10.1124/dmd.107.016261
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Legends for Figures:

Figure 1: Pathway of MDMA metabolism. Bolded arrow represents primary metabolic pathway of MDMA in humans.

Figure 2: Effects of ambient temperature on MDMA-induced thermogenesis. Data are represented as mean ± SEM (n=5-7). Abscissa represents time after MDMA (1.5 mg/kg, IV) administration and ordinate represents core temperature. Filled symbols indicate significantly different ($p<0.05$) from baseline (0 min). $^{\#}$ indicates significantly different ($p<0.05$) from 24°C baseline.

Figure 3: Effects of ambient temperature on MDMA disposition. Data are represented as mean ± SEM (n=5-7). Abscissa represents time (min) after MDMA (1.5 mg/kg, IV) administration and ordinate represents plasma concentrations (ng/mL) of (A) MDMA, (B) HHMA, (C) HHA, and (D) MDA.

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Table 1: Pharmacokinetic measures (mean ± SEM; n=5-7) of MDMA and its metabolites

	18°C	**24°C**	**31°C**
MDMA			
$t_{1/2}$ (min)	~136.3 ± 24.7	96 ± 7.1	~143.7 ± 7.1
C_{max} (ng/mL)	13,535 ± 2707	8999 ± 2569	6722 ± 2726
CL (mL/min x kg)	13.4 ± 0.4	16.4 ± 3.9	15 ± 3.4
Vd_{SS} (mL/kg)	610 ± 76	1740 ± 601	2140 ± 597
$AUC_{0\text{-}240\ min}$ (ng/mL x min)	132,224 ± 27,688	113,077 ± 27,310	101,010 ± 41,313
HHMA			
C_{max} (ng/mL)	449.6 ± 39.7	653.7 ± 86.8	754.2 ± 210.7
HHMA:MDMA[a]	0.6 ± 0.19	1.18 ± 0.33	1.91 ± 0.64
$AUC_{0\text{-}240\ min}$ (ng/mL x min)	59,459 ± 7864	86,882 ± 13,186	104,503 ± 30,014
HHA			
C_{max} (ng/mL)	121.9 ± 17.8	155.7 ± 39.2	106.7 ± 16.5
HHA:MDMA[a]	0.17 ± 0.06	0.32 ± 0.13	0.21 ± 0.05
$AUC_{0\text{-}240\ min}$ (ng/mL x min)	15,825 ± 2164	18,402 ± 3450	13,651 ± 1757
MDA			
C_{max} (ng/mL)	28.2 ± 15.9	9.6 ± 1.9	12.1 ± 3.6

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MDA:MDMA[a]	0.024 ± 0.005	0.011 ± 0.002	0.015 ± 0.007[b]
$AUC_{0\text{-}240\ min}$ (ng/mL x min)	2767 ± 451[#]	1145 ± 224	942 ± 386

[a] Ratio of the metabolite ($AUC_{0\text{-}240\ min}$) to MDMA ($AUC_{0\text{-}240\ min}$)

[b] Data represent mean ± SEM (n=3)

[#] indicates significantly different ($p < 0.05$) from other ambient temperatures

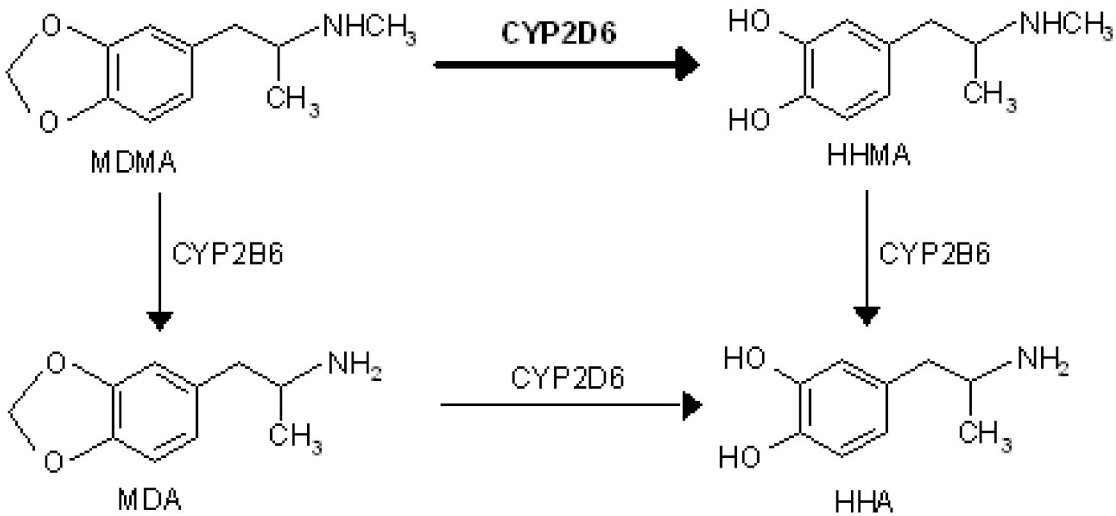
O
O
O
NHCH3
CH3
MDMA
CYP2D6
HO
HO
NHCH3
CH3
HHMA
CYP2B6
CYP2B6
O
O
O
NH2
CH3
MDA
CYP2D6
HO
HO
NH2
CH3
HHA

Figure 1

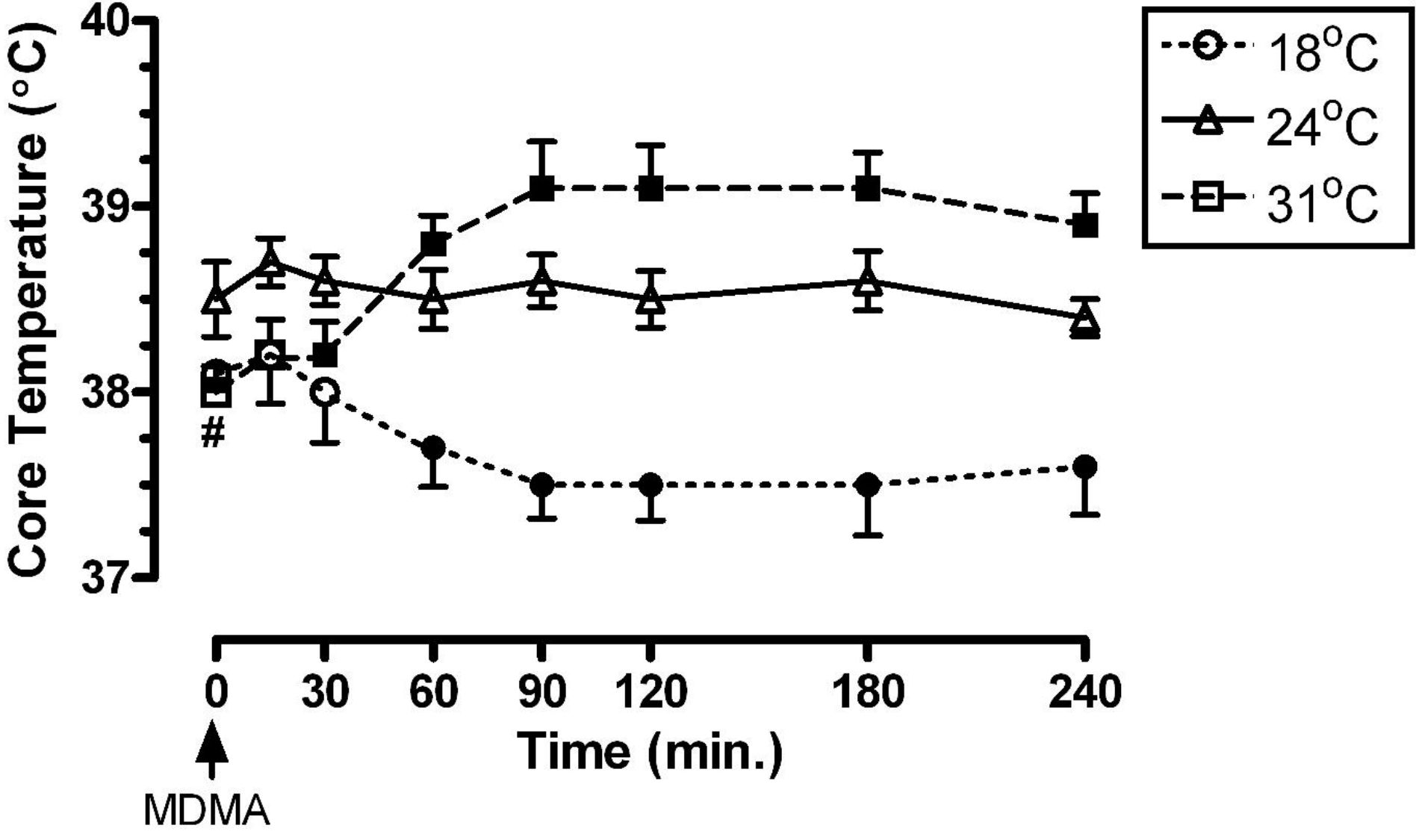
Core Temperature (°C)
40
39
38
37
#
18°C
24°C
31°C
0
30
60
90
120
180
240
Time (min.)
MDMA

Figure 2

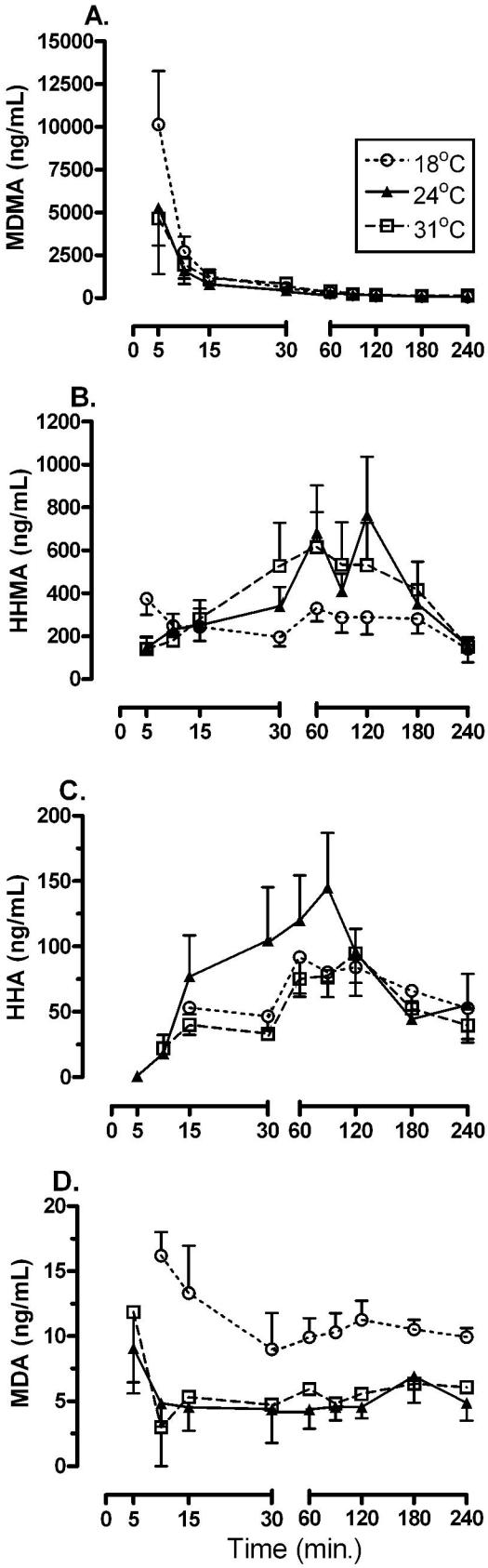
A.
MDMA (ng/mL)
15000
12500
10000
7500
5000
2500
0
18°C
24°C
31°C
0 5 15 30 60 120 180 240
B.
HHMA (ng/mL)
1200
1000
800
600
400
200
0
0 5 15 30 60 120 180 240
C.
HHA (ng/mL)
200
150
100
50
0
0 5 15 30 60 120 180 240
D.
MDA (ng/mL)
20
15
10
5
0
0 5 15 30 60 120 180 240
Time (min.)

Figure 3