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Ethanol co-administration moderates 3,4-methylenedioxymethamphetamine effects on human physiology

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Abstract

Alcohol is frequently used in combination with 3,4-methylenedioxymethamphetamine (MDMA). Both drugs affect cardiovascular function, hydration and temperature regulation, but may have partly opposing effects. The present study aims to assess the acute physiologic effects of (co-) administration of MDMA and ethanol over time. A four-way, double blind, randomized, crossover, placebo-controlled study in 16 healthy volunteers (9 male and 7 female) between the ages of 18 and 29. MDMA (100 mg) was given orally and blood ethanol concentration was maintained at pseudo-steady state levels of 0.6‰ by a three-hour 10% intravenous ethanol clamp. Cardiovascular function, temperature and hydration measures were recorded throughout the study days. Ethanol did not significantly affect physiologic function, with the exception of a short lasting increase in heart rate. MDMA potently increased heart rate and blood pressure and induced fluid retention as well as an increase in temperature. Co-administration of ethanol with MDMA did not affect cardiovascular function compared to the MDMA alone condition, but attenuated the effects of MDMA on fluid retention and showed a trend for attenuation of MDMA-induced temperature increase. In conclusion, co-administration of ethanol and MDMA did not exacerbate physiologic effects compared to all other drug conditions, and moderated some effects of MDMA alone.

Key words
alcohol; human; MDMA; physiology

Introduction

3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") is a frequently used club-drug in Western societies (Gross, 2002; Parrott, 2001). Apart from its desired effects on mood and perception, ecstasy has powerful physiologic side effects. Moreover, ecstasy users are generally multi-drug users and alcoholic beverages are commonly combined with MDMA (Barrett, *et al*., 2005; Izco, *et al*., 2007). However, the physiologic effects of this common combination, with the exception of effects on immune function (Pacifici, *et al*., 2001), have not been assessed previously.

MDMA is a potent stimulant of cardiovascular action, increasing heart rate and blood pressure (Dumont and Verkes, 2006; Green, *et al*., 2003). Disturbances in fluid homeostasis due to MDMA consumption, that is an increase of antidiuretic

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hormone concentration (ADH or vasopressin, which promotes water retention) after MDMA consumption, has been reported (Henry, *et al.*, 1998; Wolff, *et al.*, 2006). This, in turn, can lead to hyponatraemia and serious health risks (Hall and Henry, 2006; Rosenson, *et al.*, 2007). MDMA also affects temperature regulation, generally increasing body temperature. Although this has received considerable attention in the literature, the mechanism of action is controversial (Colado, *et al.*, 1995; Colado, *et al.*, 2004; Green, *et al.*, 2004a; Mechan, *et al.*, 2002; Saadat, *et al.*, 2005). Recent reports suggest the involvement of the sympathetic nervous system in MDMA-induced hyperthermia (Mills, *et al.*, 2003; Sprague, *et al.*, 2003; Sprague, *et al.*, 2005). The pharmacology of MDMA-induced hyperthermia is of special interest, as prevention of hyperthermia has been shown to diminish or even prevent MDMA-induced neurotoxicity (Malberg and Seiden, 1998; O'Shea, *et al.*, 2002).

Case reports of severe, sometimes fatal, physiologic disturbances after MDMA use, which are often facilitated by unfavorable behavior and/or circumstances, illustrate the relevance of these side effects of MDMA use (Connolly and O'Callaghan, 1999; Kalantar-Zadeh, *et al.*, 2006), although the incidence is low relative to the large population at risk (Nutt, 2006).

Drinks containing ethanol, commonly referred to as alcohol, are regularly used in social settings. Ethanol is an allosteric modulator of many transmembrane receptors (Pohorecky and Brick, 1988), but functionally it acts foremost as a CNS depressant, depressing both excitatory and inhibitory postsynaptic potentials by potentiating the action of GABA at the GABAa receptor (Suzdak, *et al.*, 1988). Although the chronic effects of ethanol on physiologic function have been assessed frequently, reports of acute physiological effects of ethanol are less frequent. Contrary to the effects of chronic ethanol exposure, which increases blood pressure (Kodavali and Townsend, 2006), acute ethanol administration moderately lowers blood pressure and increases heart rate (Pohorecky and Brick, 1988; Silva, *et al.*, 2004; Tawakol, *et al.*, 2004). Ethanol also affects hydration regulation (e.g., promotes diuresis). Although some reports suggested that ethanol attenuates blood ADH concentration (Madeira and Paula-Barbosa, 1999), others did not find an effect of ethanol administration on ADH levels (Rivier and Lee, 1996; Silva, *et al.*, 2004). Effects of ethanol on body temperature also remain poorly understood. Generally, ethanol has been found to lower body temperature, tentatively explained by its vasodilatory effects. Recent studies suggest that the effects of ethanol on thermoregulatory behavior are major contributors to the hypothermic effect of ethanol in humans (Turek and Ryabinin, 2005; Yoda, *et al.*, 2005).

As both substances have distinct and possibly opposite actions on physiologic function, we hypothesized that moderate ethanol intake may ameliorate the effects of MDMA. Co-administration of ethanol with MDMA may ameliorate MDMA-induced water retention by promoting diuresis, and as such protect against hyponatraemia and its consequences. Cardiovascular distress after MDMA may be enhanced by co-administration of ethanol, as ethanol acutely increases heart rate. On the other hand, MDMA-induced cardiovascular distress may also be attenuated via ethanol's central depressant effects, which may attenuate sympathetic drive of cardiovascular function. The hypothermic effect of ethanol may offset MDMA-induced temperature increase when co-administered, which in turn may diminish MDMA's neurotoxic potential.

Materials and methods

Study design

This study used a four-way, double blind, randomized, cross-over, and placebo-controlled design. Sixteen volunteers were randomly assigned to one of the four treatment sequences. Each volunteer received a capsule containing either 100 mg MDMA or placebo and an ethanol or placebo infusion (target blood alcohol concentration of 0.6‰) with a washout of 7 days between each treatment.

Study outline

Subjects were admitted to each study day after a urinary drug check (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines, and delta-9-tetrahydrocannabinol, AccuSign®, Princeton BioMeditech, Princeton, USA) (drug use was not allowed 14 days prior to the first study day until study completion) and the recording of possible signs and symptoms of health problems. A light breakfast was offered. Drug administration was scheduled at 10:30 h and the ethanol infusion was started at 11:00 h. A 30 min lunch break was scheduled 210 min after drug administration. Outcome measures were assessed before MDMA administration and at 30, 90, 150, 240, 300, and 360 min post drug administration and consisted of cardiovascular function assessed by heart rate, systolic-, and diastolic blood pressure measurements using a Datascope® Accutorr Plus™ cardiovascular monitor and temperature measurements using a Braun® type 6021 ThermoScan. Room temperature was kept at 22°C. Blood was collected for the measurement of MDMA, antidiuretic hormone (ADH), sodium, norepinephrine (NE), and epinephrine (E) plasma concentration. The latter two were collected at baseline, 60 and 150 min after drug administration. Subjects received lunch at 14:00 h and were sent home at 17:00 h after a medical check up.

Subjects

Sixteen healthy volunteers (9 male and 7 female), regular users of ecstasy (at least eight exposures in the last two years) and alcohol (at least one exposure per week), 22.1 ± 2.9 (mean ± SD) years of age (range 18–29), and within 80–130% of their ideal body weight were recruited through advertisement on the internet and at local drug testing services. Lifetime ecstasy exposure was on average 95 (SD = 138; range 14–431).

More detailed demographic data have been reported elsewhere (Dumont, *et al*., 2008). Exclusion criteria included pregnancy, (history of) psychiatric illness, use of over-the-counter medication within 2 months prior to the study start, (history of) treatment for addiction problems, (familial or personal history of) schizophrenia, excessive smoking (>10 cigarettes/day), and orthostatic dysregulation. Physical and mental health was determined by assessment of medical history, a physical-, and ECG examination as well as standard haematological and chemical blood examinations. None of the subjects screened for study participation showed signs of cardiovascular disturbances or (a history of) psychiatric illness. The local Medical Ethics Committee approved the study. All subjects gave their written informed consent before participating in the study, and were paid for their participation. One subject had a mild adverse reaction (local vascular reaction) to the ethanol infusion and one subject did not refrain from drug use, both (1 male and 1 female) were excluded from further participation and results obtained from these subjects were not included in the final data analysis.

Ethanol clamping

Ethanol (or glucose 5% as its placebo) was administered continuously by IV infusion of 10% ethanol in 5% glucose solution, aimed to maintain an ethanol blood concentration of 0.6‰ for three hours. The alcohol clamp was targeted at 0.6‰, the equivalent of approximately 2–3 alcoholic beverages. This concentration is just above the legal limit for traffic participation in many Western countries and commonly used in social settings, as it is considered to be a safe and relatively moderate level, despite significant CNS effects (Amatsaleh, *et al*., 2006b). The infusion rate was calculated using frequent breath alcohol concentration measurements, according to a previously designed algorithm (Amatsaleh, *et al*., 2006a). Breath alcohol concentration was assessed using a HONAC AlcoSensor IV® Intoximeter. The process was semi-automated using a computer spreadsheet program, which uses changes in the measured breath alcohol concentrations to calculate the infusion rate that is needed to maintain the ethanol level at 0.6‰. The operator of the breath alcoholmeter and the ethanol infusion pump was unblinded for alcohol-treatment, but did not communicate with the study team or the subject about the results at any stage during the trial. A sham-procedure including a mock-spreadsheet was used on ethanol-placebo-occasions.

MDMA

MDMA (or matched placebo) was given orally as a capsule in a single dose of 100 mg. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland, and encapsulated according to Good Manufacturing Practice by the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre. MDMA (100 mg, orally) is a relevant dose in the range of normal single recreational dosages. Previous experiments in humans used doses up to 150 mg without serious adverse events.

Analytical methods

Reagents All reagents were of analytical grade.

MDMA analysis Plasma samples were stored frozen at −70°C until the time of analysis. An HPLC–diode array detection method was employed to measure MDMA plasma concentration (Dumont, *et al*., 2008).

Hormone analysis ADH was measured by an in-house radioimmunoassay RIA employing 125I-labelled ADH and an antibody raised against arginine-vasopressin, performed after prepurification of ADH by means of Sep-Pak C18 columns. The average recovery was 75 ± 8%. Within- and between-assay CVs were as follows: 7.1 and 14.0% at 2.6 pmol/l, 3.4 and 8.5% at 5.0 pmol/l and 4.0, and 9.4% at 8.9 pmol/l.

Plasma NE and E concentration was measured by a sensitive and specific HPLC with fluorometric detection as described previously (Willemsen, *et al*., 1995). Blood samples were collected after the subject had remained in a seated position for at least 15 minutes and were processed within 30 min after collection.

Statistical analyses

Pharmacodynamic parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED; SAS 9.1.3 for Windows, SAS Institute, Inc., Cary, NC) with treatment, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects are reported as the contrasts between the four treatments where the average of the measurements up to last time point was calculated within the statistical model. Contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05.

Temperature measurements were also converted to a composite measure (TAUC) as described by Miller and O'Callaghan (2003). This composite measure represents the area under the curve of a plot of temperature (°C) and time (min) and has units of °C min. Statistical evaluation of TAUC was performed using the GLM Repeated Measures Analysis of Variance in SPSS 12 for windows. The relationships between MDMA dose corrected for body weight and MDMA maximal plasma concentration and between MDMA dose corrected for body weight and heart rate were statistically evaluated using a linear regression analysis in SPSS 12 for Windows.

Results

Only significant results are mentioned in this section unless noted otherwise. Main effects of treatment, time, and treatment by time as well as drug condition comparisons are summarized in Table 1. For the drug condition comparisons, (percentual) change, 95% confidence interval (95% CI) and corresponding *P*-values are reported.

Kinetics

Mean MDMA maximal plasma concentration (C_{max}) was 202.5 ng/ml (SD = 74.1 ng/ml) 150 min after drug administration, mean ethanol steady-state concentration was 0.56‰ (SD = 0.06‰). A positive linear relationship was found for MDMA dose corrected for body weight and MDMA C_{max} ($R = 0.85$, $P < 0.001$, see Figure 1). Ethanol co-administration did not affect this relationship. MDMA and ethanol kinetics in time are reported elsewhere (Dumont, *et al*., accepted for publication).

Cardiovascular function

Heart rate was increased in all drug conditions when compared to placebo as shown in Figure 2A. Co-administration of MDMA and ethanol did not increase heart rate when compared to the MDMA alone condition. Maximal heart rate increase was correlated with MDMA dose corrected for body weight and showed a positive linear dose-response curve ($R = 0.69$, $P < 0.001$).

Systolic and diastolic blood pressure showed a similar response to drug administration in time and were increased after MDMA as well as MDMA and ethanol (co-) administration compared to the placebo and ethanol condition, Figure 2B shows the mean arterial pressure.

NE and E concentrations

NE levels, shown in Figure 3A, were increased after MDMA administration when compared to the placebo and ethanol condition. E levels, shown in Figure 3B, were increased after MDMA as well as MDMA and ethanol (co-) administration when compared to the ethanol and placebo condition. Co-administration of ethanol with MDMA did not increase NE levels when compared to placebo. Ethanol alone did not affect NE concentrations when compared to placebo either. Compared to the ethanol condition, co-administration of ethanol and MDMA increased NE concentrations.

Hydration

ADH (or vasopressin) plasma concentrations are shown in Figure 4 and were increased after MDMA administration compared to placebo. Ethanol administration alone did not affect ADH levels compared to placebo. Co-administration of ethanol with MDMA reversed the MDMA-induced increase of ADH concentrations to levels comparable to placebo.

Sodium plasma concentrations were decreased after MDMA administration compared to all other conditions. Co-administration of ethanol with MDMA did not significantly affect sodium plasma concentrations compared to the placebo condition. Ethanol alone also did not have an effect on sodium plasma concentrations.

Temperature

Temperature, shown in Figure 5A, increased significantly after MDMA administration when compared to the placebo (by 0.4°C on average) as well as compared to the ethanol condition. Ethanol did not affect temperature significantly when compared to any drug condition, although there was a trend ($P = 0.09$) for attenuation of the MDMA effect on temperature during co-administration of MDMA and ethanol. Temperature data was also converted to AUC data (TAUC, Figure 5B). Following a significant main effect of drug condition ($F(3,11) = 4.049$, $P = 0.036$), subsequent pairwise comparisons showed a significant increase in TAUC after MDMA administration compared to placebo ($P = 0.015$), ethanol ($P = 0.018$), and MDMA and ethanol co-administration ($P = 0.016$). Other comparisons did not reveal significant differences between drug conditions. In other words, co-administration of ethanol with MDMA reversed the MDMA-induced increase of TAUC to levels comparable to placebo.

Discussion

This study confirms previous findings that MDMA exerts potent stimulatory effects on the human cardiovascular system, induces an increase in temperature and a disturbance of water homeostasis. Ethanol single administration did not affect the physiological parameters investigated in this study, with the exception of a mild increase in heart rate. Co-administration of ethanol with MDMA did not exacerbate MDMA-induced stimulation of cardiovascular measures, and moderated the effect of MDMA on temperature and water homeostasis. Thus, co-administration of low-dose ethanol, that is, the equivalent of 2–3 alcoholic beverages, with MDMA attenuates some and does not exacerbate any of MDMA's physiologic effects.

A relatively wide range (1.1–2.2 mg/kg) of MDMA dose corrected for body weight was administered in the current study. Our current findings show a significant positive linear relationship between MDMA dose corrected for body weight and C_{max}, where an increase in dose of 0.1 mg/kg elevated MDMA C_{max} with approximately 20 μg/l (Figure 1). Ethanol co-administration did not affect this relationship. A previous study reported a nonlinear dose-response relationship for MDMA dose versus MDMA peak blood concentrations (de la Torre, *et al*., 2000). Although this study assessed the C_{max} of separate doses (50, 100, and 150 mg), the sample size was

Table 1 *P*-values for treatment, time, and treatment by time effects ($N = 14$). Comparisons of drug conditions show (percentual) change, 95% confidence intervals (between brackets) and *P*-values.

Measure	Treatment Effect	Time Effect	Treatment * Time Interaction	Ethanol vs. Plac	MDMA vs.Plac	MDMA+Eth vs. Plac	MDMA+Eth vs. Ethanol	MDMA+Eth vs. MDMA	MDMA vs. Ethanol
E and NE concentration									
E (nmol/l)	<.0001	NS	NS		306.6% (197.1%,456.4%) $P \leq .0001$	293.0% (187.1%,438.1%) $P \leq .0001$	212.8% (128.5%,328.2%) $P \leq .0001$		223.6% (136.5%,342.6%) $P \leq .0001$
NE (nmol/l)	0.0014	NS	0.0153		22.5% (4.2%,44.1%) $P = 0.0155$		25.2% (6.6%,47.2%) $P = 0.0077$		40.3% (19.4%,65.0%) $P = 0.0002$
Cardiovascular parameters									
HR (bpm)	<.0001	<.0001	<.0001	9.0% (2.4%,16.1%) $P = 0.0085$	25.7% (18.0%,33.9%) $P \leq .0001$	31.7% (23.7%,40.2%) $P \leq .0001$	20.8% (13.5%,28.7%) $P \leq .0001$		15.3% (8.3%,22.7%) $P \leq .0001$
SBP (mm Hg)	<.0001	0.0002	NS		11.2% (7.2%,15.4%) $P \leq .0001$	12.0% (7.9%,16.2%) $P \leq .0001$	10.9% (6.8%,15.0%) $P \leq .0001$		10.1% (6.1%,14.3%) $P \leq .0001$
DBP (mm Hg)	<.0001	<.0001	NS		10.9% (5.9%,16.2%) $P \leq .0001$	10.9% (5.9%,16.2%) $P \leq .0001$	12.3% (7.3%,17.6%) $P \leq .0001$		12.3% (7.2%,17.5%) $P \leq .0001$
Hydration parameters									
ADH (pmol/l)	0.0286	NS	NS		22.2% (2.5%,45.7%) $P = 0.0269$			−23.1% (−35.5%,−8.4%) $P = 0.0044$	
Na (mmol/l)	0.0132	<.0001	<.0001		−0.9% (−1.6%,−0.2%) $P = 0.0128$				−1.1% (−1.8%,−0.4%) $P = 0.0023$
Temperature									
Temperature (°C)	0.0029	NS	0.0034		0.4°C (0.2,0.7) $P = 0.0021$				0.5°C (0.2,0.8) $P = 0.0008$

E, epinephrine; NE, norepinephrine; ADH, antidiuretic hormone; Plac, placebo; Eth, ethano.

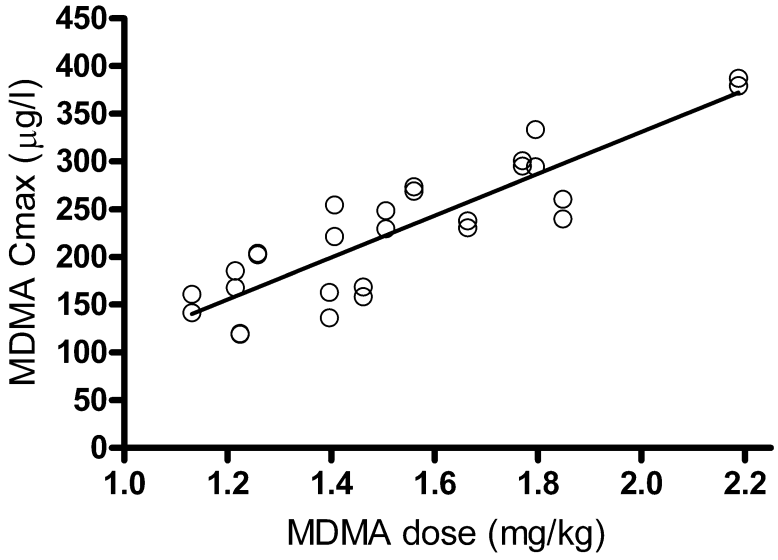

Figure 1 Effect of MDMA dose corrected for body weight on MDMA maximal plasma concentration (C_{max}). A significant linear correlation between dose (in mg/kg body weight) and C_{max} (in µg/l) was found ($R = 0.851$, $P < 0.001$).

relatively small ($N = 6$, two subjects per dose tested) and doses were not corrected for body weight (reported weight range was 66–83 kg).

A significant, positive linear dose-response relationship was found for the effect of MDMA dose (corrected for body weight) on heart rate, where an increase of 0.1 mg/kg induced an average increase in heart rate of 4.2 bpm. Ethanol co-administration did not affect this relationship. Although ethanol single administration led to a modest increase in heart rate, co-administration of ethanol and MDMA did not show an additive effect on heart rate.

Blood pressure was increased after MDMA as well as after MDMA and ethanol co-administration (see Figure 2B). The lack of ethanol effects on blood pressure is in contrast with previous findings, which reported a moderate decrease of blood pressure after ethanol administration (Pohorecky and Brick, 1988; Silva, *et al*., 2004; Tawakol, *et al*., 2004). The ethanol administration route (infusion of 10% ethanol solution over three hours) may have counteracted the effects of ethanol on blood pressure.

Hyponatraemia has been suggested to be a potentially fatal side effect of MDMA use, likely due to MDMA-induced increase in ADH concentration (Hartung, *et al*., 2002; Henry, *et al*., 1998). In line with this suggestion, we report a decreased sodium plasma concentration and increased ADH concentration after MDMA administration. Co-administration of ethanol with MDMA attenuated the MDMA-induced increase in ADH concentration (Figure 4). After co-administration, sodium concentration did not differ from placebo or from the MDMA condition. Although the effects reported here are relatively small and are unlikely to be of clinical relevance, the circumstances in which MDMA is typically used may exacerbate these effects (Kalantar-Zadeh, *et al*., 2006) (crowded, high ambient temperature clubs in combination with vigorous dancing as well as excessive water intake stimulated by public education). Ethanol single administration did not affect these measures. Previous reports have shown that ethanol can attenuate ADH release (Madeira and Paula-Barbosa, 1999), although others did not find an effect of ethanol administration on ADH levels (Rivier and Lee, 1996; Silva, *et al*., 2004). Possibly, the continuous administration of low levels of ethanol is insufficient to disturb ADH regulation *per se*, whereas the increased ADH concentration after MDMA allowed ethanol to demonstrate its attenuating effect on ADH release.

Body temperature (Figure 5) has been shown to robustly increase after MDMA administration in animals (Green, *et al*., 2005). In the current (human) study, MDMA increased body temperature slightly but significantly with an average maximal increase of 0.4 degrees Celsius. Although this effect is very moderate in comparison to the animal data, the effects on temperature regulation may be of significance in recreational MDMA use settings. These environments are assumed to be crowded and to have a high ambient temperature. Combined with vigorous dancing, these factors may facilitate the MDMA-induced increase of body temperature beyond the effect size observed in the current, strictly controlled laboratory study (Freedman, *et al*., 2005; Parrott, *et al*., 2006; Williams, *et al*., 1998). Two naturalistic studies assessed the effects of (among others) MDMA on body temperature during a "club night" (Cole, *et al*., 2005; Irvine, *et al*., 2006). Both studies did

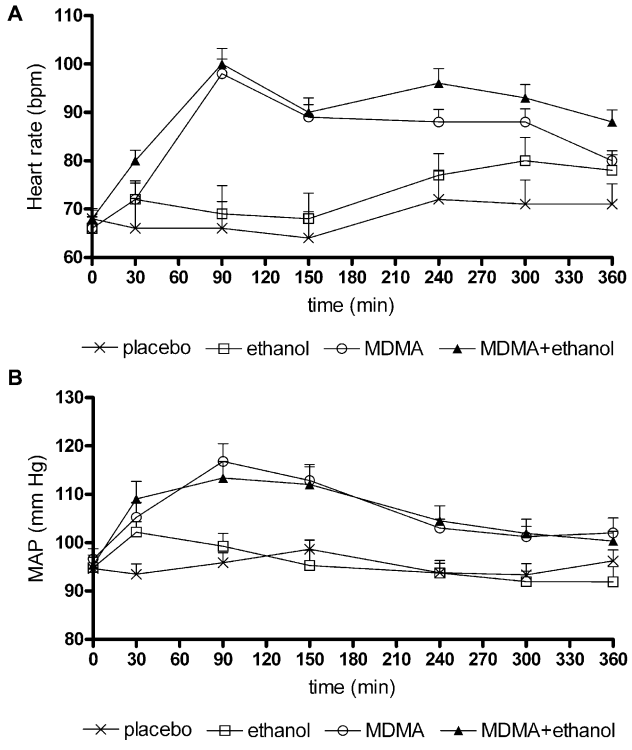

Figure 2 Cardiovascular effects. A: Heart rate (in bpm) per drug condition (mean, s.e.m.). Heart rate was increased in all drug conditions compared to placebo. Compared to the ethanol condition, MDMA administration as well as co-administration of MDMA and ethanol increased heart rate (all comparisons $P < 0.01$). B: Mean arterial pressure (MAP, in mm Hg) per drug condition (mean, s.e.m.). Systolic and diastolic blood pressure showed a similar response to drug administration, both were increased in the MDMA as well as the MDMA and ethanol condition compared to the placebo and the ethanol condition (all comparisons $P < 0.01$).

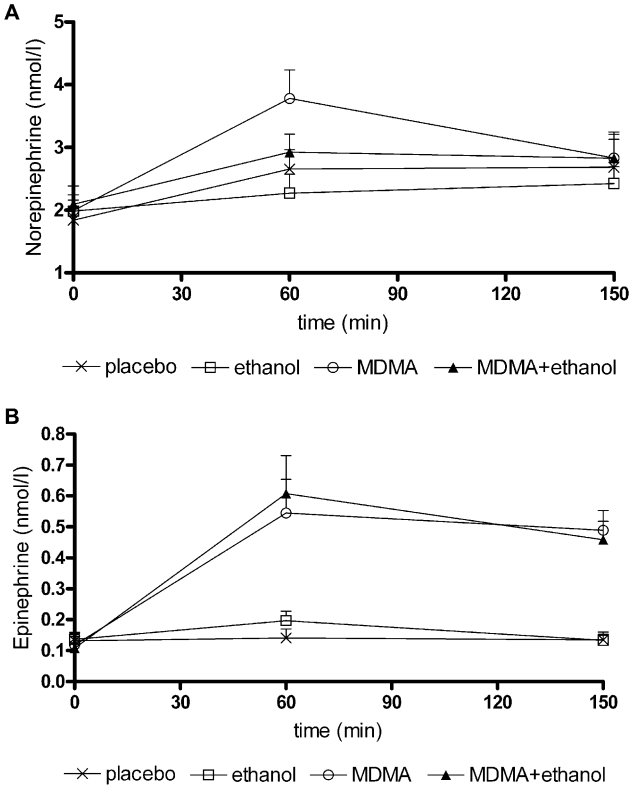

Figure 3 NE and E plasma concentrations. A: NE plasma concentration (in nmol/l) per drug condition (mean, s.e.m.). Norepinephrine plasma concentrations were increased after MDMA administration compared to the placebo ($P = 0.02$) and the ethanol condition ($P < 0.01$). Compared to the ethanol condition, MDMA administration as well as co-administration of MDMA and ethanol increased NE concentrations (all comparisons $P < 0.01$). Co-administration of ethanol with MDMA did not increase NE levels compared to placebo. B: E plasma concentration (in nmol/l) per drug condition (mean, s.e.m.). E levels were increased after MDMA as well as MDMA and ethanol (co-) administration compared to the ethanol and the placebo condition (all comparisons $P < 0.01$).

not find a significant rise in body temperature in users of psychostimulants (among which MDMA). However, all psychostimulant users in the study of Cole, *et al.* (2005) reported the co-use of alcohol, thus confirming our current findings. The study by Irvine, *et al.* (2006), which showed a trend for increased body temperature, did not assess alcohol co-use, although the authors did note that all participants were regular users of alcohol.

In rats, MDMA-induced hyperthermia has been shown to be mediated by the sympathetic nervous system, more specifically via α1-mediated vasoconstriction and β3-mediated thermogeneration in rats (Blessing, 2005; Mills, *et al.*, 2003; Mills, *et al.*, 2007; Sprague, *et al.*, 2005). In the current study, MDMA indeed potently increased E and NE plasma concentrations (Figure 3). Ethanol co-administration attenuated the MDMA-induced NE (but not E) increase as well as MDMA-induced temperature increase. Moreover, the above mentioned report by Sprague, *et al.* (2005) showed that the blockade of either heat generation (mediated by the β_3 receptor) or blockade of vasoconstriction (mediated by the α_1 receptor) could only reduce hyperthermia by approximately 50%. In the current study, temperature after MDMA and ethanol co-administration did not differ from placebo and showed a trend for attenuation of MDMA-induced temperature increase. This is in line with the abovementioned report, as ethanol co-administration attenuated NE concentration, effectively diminishing heat generation (the β_3 receptor shows a higher affinity for NE over E), although ethanol co-administration did not reduce the elevated E plasma concentrations. As the α_1 receptor, mediating vasoconstriction, has equal affinity for NE and E, the increased E concentration is likely to powerfully maintain vasoconstriction, and thus impair heat dissipation,

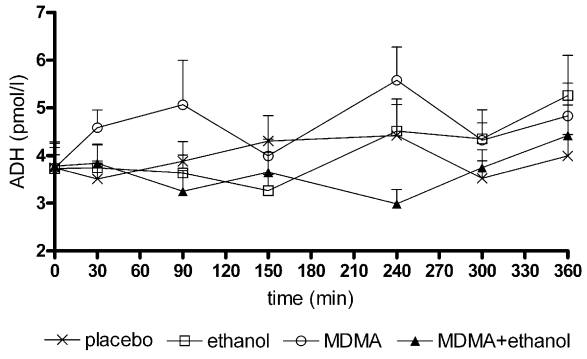

Figure 4 ADH plasma concentrations (in pmol/l) per drug condition (mean, s.e.m.). ADH plasma concentrations were increased after MDMA administration compared to placebo ($P = 0.03$). Co-administration of ethanol with MDMA reversed the MDMA-induced increase of ADH concentrations ($P < 0.01$).

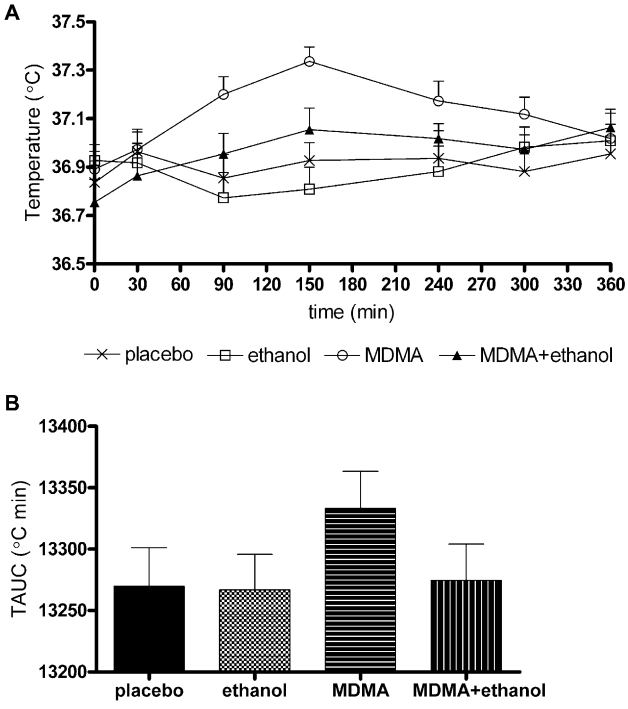

Figure 5 Temperature effects. A: Temperature (in °C) per drug condition (mean, s.e.m.). Temperature was increased in the MDMA condition when compared to the placebo and the ethanol condition. There was a trend ($P = 0.09$) of attenuation of the MDMA effect on temperature by co-administration of ethanol with MDMA. B: Temperature AUC effects (TAUC in °C min) per drug condition (mean, s.e.m.). TAUC was increased in the MDMA condition when compared to the placebo and ethanol condition (both comparisons $P = 0.02$). Ethanol co-administration attenuated MDMA's TAUC effects ($P = 0.02$).

confirming the findings of Sprague, *et al.* (2005) in humans. The attenuation of NE output did not affect cardiovascular measures, although the reduced NE concentration should reduce vasoconstriction (mediated by NE) and thus lower blood pressure. The potent stimulatory effects on heart rate (mainly mediated by E) have probably counteracted any effect of the reduced concentration on blood pressure (Figure 2).

Although MDMA's neurotoxic potential in humans is still a matter of debate (Gouzoulis-Mayfrank and Daumann, 2006), MDMA-induced temperature increase is of particular interest as its prevention has been shown to be an effective way of reducing or even preventing MDMA-induced neurotoxicity in animal studies (Goni-Allo, *et al.*, 2007; Malberg and Seiden, 1998; O'Shea, *et al.*, 2002). However, our findings suggest that low-dose ethanol co-administration, by attenuating sympathetic output, may moderate MDMA-induced neurotoxicity. Although this provides interesting avenues for future studies regarding MDMA-induced neurotoxicity, the current study did not assess these effects under the circumstances where temperature increase may become clinically relevant. In fact, a recent study in rats reported that ethanol did not attenuate MDMA-induced temperature increase in high ambient temperature (Cassel, *et al.*, 2007). Moreover, as ethanol is able to increase hydroxyl radical formation, higher doses of ethanol may potentiate MDMA-induced oxidative stress, and thus neurotoxicity, as suggested by a recent study that showed that repeated pre-exposure to high doses of ethanol exhausted CNS antioxidant resources and potentiated the neurotoxic effects of a subsequent dose of MDMA (Izco, *et al.*, 2007).

Our results should be considered explorative because of some limitations of our study design. First, the attenuating effects of ethanol co-administration on temperature and hydration did not reach statistical significance when directly compared to the single MDMA condition (with the exception of ADH and TAUC effects). It is likely that our study did not have sufficient power to statistically distinguish between these relatively small effects, although as discussed these effects may be enhanced and become relevant under more naturalistic conditions. Second, we assessed the effects of a single dose of MDMA and subsequent ethanol administration, and effects may differ depending on the dose assessed and the sequence of drug administration. Moreover, effects were assessed at a single ambient temperature of 22°C, and different ambient temperatures may induce different effects. In general, the circumstances in which these substances are normally used cannot be fully recreated in the laboratory. These circumstances, along with the different expectations and behavior, likely influence the effects of MDMA (Sumnall, *et al.*, 2006). Thus, further research should investigate the effects of the surroundings that ecstasy users are exposed to while being intoxicated, although such studies face considerable issues regarding feasibility (Irvine, *et al.*, 2006). Such studies should also assess the effects of different doses of ethanol and MDMA on physiologic function to corroborate our suggestions. Lastly, the difference in

ethanol administration and resulting kinetics may have influenced our results, as it has been shown that some ethanol effects (i.e. sedation) manifest only during ascending or descending BAC (Pohorecky and Brick, 1988).

As studies investigating the therapeutic potential of MDMA are emerging (Parrott, 2007; Sessa, 2007; Sessa and Nutt, 2007), information on how to treat and possibly even prevent side effects of MDMA in clinical studies appears vital. Our results suggest that antagonism of the sympathetic nervous system during MDMA use may diminish temperature increase, and, although speculative, thereby may diminish the possibility of neurotoxicity. Moreover, the cardiovascular distress induced by MDMA may also be effectively treated or prevented via this intervention. Last, careful management of fluid intake may effectively manage the symptoms of inappropriate increase in ADH concentration under controlled circumstances.

In conclusion, co-administration of ethanol and MDMA did not exacerbate physiologic effects when compared to all other drug conditions, and moderated some effects of MDMA alone. It should be stressed that these findings are only valid for the relatively low dose of ethanol (0.6‰ or 2–3 alcoholic beverages) as employed in the current study. Although the effects observed in this study are considered subtle, they demonstrate that MDMA and ethanol disregulate physiological systems that are particularly important during the typical circumstances in which MDMA is used (Cassel, *et al*., 2007; Cornish, *et al*., 2003; Green, *et al*., 2004b; Hargreaves, *et al*., 2007).

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