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ORIGINAL INVESTIGATION

Interactions between ethanol and cocaine, amphetamine, or MDMA in the rat: thermoregulatory and locomotor effects

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Abstract
Rationale (±)-3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is often taken recreationally with ethanol (EtOH). In rats, EtOH may potentiate MDMA-induced hyperactivity, but attenuate hyperthermia.
Objective Experiment 1 compared the interactions between EtOH (1.5 g/kg) and MDMA (6.6 mg/kg) with EtOH + cocaine (COCA; 10 mg/kg) and EtOH + amphetamine (AMPH; 1 mg/kg) on locomotor activity and thermoregulation. Experiment 2 used a weaker dose of MDMA (3.3 mg/kg) and larger doses of COCA (20 mg/kg) and AMPH (2 mg/kg).
Materials and methods Drug treatments were administered on four occasions (2, 5, and 2 days apart, respectively; experiment 1) or two (2 days apart; experiment 2).
Results All psychostimulants increased activity, and EtOH markedly increased the effect of MDMA. AMPH alone-related hyperactivity showed modest sensitization across treatment days, while MDMA + EtOH activity showed marked sensitization. AMPH, COCA, and MDMA induced hyperthermia of comparable amplitude (+1 to +1.5°C). Co-treatment with EtOH and AMPH (1 mg/kg) or COCA (10 mg/kg) produced hypothermia greater than that produced by EtOH alone. Conversely, EtOH attenuated MDMA-related hyperthermia, an effect increasing across treatment days. These results demonstrate that the interaction between MDMA and EtOH may be different from the interaction between EtOH and AMPH or COCA.
Conclusion Because of potential health-related consequences of such polydrug misuse, it is worth identifying the mechanisms underlying these interactions, especially between EtOH and MDMA. Given the different affinity profiles of the three drugs for serotonin, dopamine, and norepinephrine transporters, our results appear compatible with the possibility of an important role of serotonin in at least the EtOH-induced potentiation of MDMA-induced hyperlocomotion.

Keywords Abuse · Alcohol · Amphetamine · Cocaine · Locomotor activity · Rat · Temperature

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Introduction

3,4-methylenedioxymethamphetamine (MDMA), an amphetamine derivative also called ecstasy, is a popular recreational drug used mostly by young people in dance club and rave cultures (Green et al. 1995, 2003; Schifano 2004). In rodents and primates, MDMA causes a rapid release of serotonin and dopamine, has psychostimulant effects, and induces hyperthermia that can occasionally be fatal (Schifano 2004). In rats and primates, MDMA may produce long-term serotonergic toxicity (Cole and Sumnall 2003; Taffe et al. 2001; Xie et al. 2006). Interspecies differences, however, have been described in terms of physiological, behavioral, and even toxicological effects of MDMA (Easton and Marsden 2006). For example,

primates do not respond to MDMA by hyperlocomotion (Taffe et al. 2006). Furthermore, MDMA toxicity is dopaminergic in mice, but serotonergic in rats and primates (Colado et al. 2004).

In humans, recreational polydrug use is quite common (Pedersen and Skrondal 1999). Indeed, MDMA is frequently taken in combination with other drugs such as, cannabis, amphetamine, or cocaine (Scholey et al. 2004). One of the drugs most frequently combined with MDMA is ethanol (EtOH); EtOH is easily available and legal in most countries (Scholey et al. 2004; Lora-Tamayo et al. 2004). In rats tested at ambient temperatures of 21–23°C, EtOH was found to dramatically potentiate the hyperlocomotion induced by MDMA, but surprisingly, to attenuate its hyperpyretic effects (Cassel et al. 2004, 2005). In the present study, we wondered if the effects that we observed with EtOH and MDMA were unique or whether we would observe the same behavioral and thermoregulatory effects with other psychostimulants acting on monoaminergic systems, even with different mechanisms of action. Like MDMA, cocaine (COCA) or amphetamine (AMPH) both induce ambient temperature-dependent hyperthermia (e.g., (Ansah et al. 1996; Gonzalez 1993; Borbely et al. 1974), although the affinity of these drugs for the different monoamine transporters is not comparable, whether in humans or rodents (e.g., (Han and Gu 2006). Furthermore, previous studies highlighted differences in the thermoregulatory effects of MDMA and AMPH or derivates (e.g., (Jaehne et al. 2005)), suggesting that the effects of interactions between various psychostimulants and EtOH could also be quite different. Thus, in our first experiment, we compared the thermoregulatory and locomotor stimulatory effects of MDMA (6.6 mg/kg, i.p.), D-amphetamine (AMPH; 1 mg/kg, i.p.), or cocaine (COCA; 10 mg/kg, i.p.) coadministered or not with EtOH (1.5 g/kg, i.p.) in young adult male rats. The doses were chosen based on preliminary experiments carried out in our laboratory to produce equivalent levels of hyperthermia among the three agents. In a second experiment, we measured the thermoregulatory and locomotor effects at a lower dose of MDMA (3.3 mg/kg, i.p.) and at higher doses of AMPH (2 mg/kg, i.p.) and COCA (20 mg/kg, i.p.) combined with 1.5 g/kg EtOH.

Materials and methods

In our first experiment, the drugs/combination were injected on four occasions as depicted in Fig. 1. For the second experiment, temperature and locomotor activity were recorded on D4 and D6 only.

Subjects

For the first experiment, 115 male Long–Evans rats (aged of 3 months; Centre d'Elevage R. Janvier, Le Genest-St-Isle, France) were used. They were housed individually in transparent Makrolon cages (42×26×15 cm) under controlled temperature (23°C±1°C) and a 12/12 h light/dark cycle (lights on at 7:00 A.M.). Food and water were provided ad libitum, including during activity or temperature

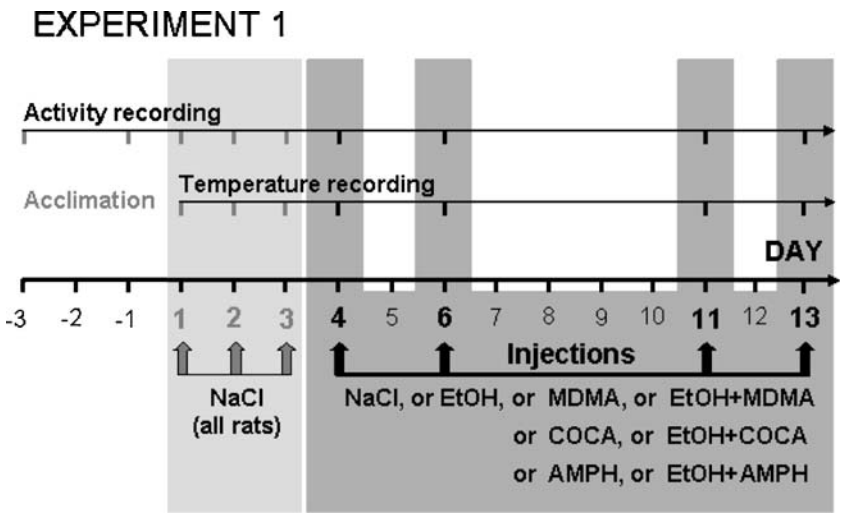

Fig. 1 Schematic representation of the time table of our protocol in experiment 1. All rats were first exposed to the test conditions of activity (*top*) or temperature (*below*) recording (days −3 and −1). They were subsequently (days 1–3) injected with saline (NaCl 0.9%, 7.5 ml/kg, i.p.) to habituate them to all manipulations accompanying the injection procedure. Over the next 10 days, on four occasions (days 4, 6, 11, and 13), they were injected with ethanol (1.5 g/kg, i.p.), amphetamine (AMPH; 1 mg/kg, i.p.), cocaine (COCA; 10 mg/kg, i.p.), MDMA (6.6 mg/kg, i.p.), ethanol + AMPH, ethanol + COCA, or ethanol + MDMA (same doses as for the single injections), or with saline as the control for normal activity (NaCl 0.9%, 7.5 ml/kg). Separate sets of rats, which were all naïve to ethanol or any of the other drugs, were used for determination of locomotor activity and body temperature changes. In the second experiment, only two injections were performed (D4 and D6)

recording experiments. After arrival, the animals were allowed to acclimate to the laboratory for 1 week, during which they were handled for 5 min daily. At the end of that week, they were randomly assigned to one of two experiments, one in which locomotor activity was assessed (n= 64) and one in which body temperature was measured (n= 51). All experimental procedures were conducted in conformity with both the national institutional guidelines (council directive 87848, October 19, 1987, *Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale*; permission 67-215 to J-C.C. and 67-217 to C.K; other authors were supervised by J-C.C. under his authority) and the international guidelines (NIH publication, 86-3, revised 1985). All efforts were made to reduce the number of animals to a minimum within statistical constraints.

For the second experiment, we used 186 rats, 42 for locomotor activity recordings and 40 for temperature measurements with the low dose of MDMA in combination or not with EtOH treatment, along with the corresponding controls (NaCl, EtOH). Rats, 104, were used for assessing the locomotor and pyretic effects of the high doses of AMPH or COCA, in combination or not with EtOH, along with the corresponding controls (NaCl, EtOH). The provider, strain, age, sex, and housing conditions were exactly the same as for the first experiment.

Pharmacological treatments

The EtOH solution (20% *w*/*v*) was prepared from absolute EtOH diluted in 0.9% NaCl. (±)-3,4-methylenedioxymethamphetamine (NIDA, USA), COCA hydrochloride (NIDA, USA), and D-AMPH sulfate (Sigma, Saint-Louis, USA) were diluted in 0.9% NaCl. All drugs, whether administered alone or in combination, and the NaCl solution, were injected intraperitoneally (i.p.) in a volume of 7.5 ml/kg, 1 to 5 min before activity recording was started, or about 60 min (see below for detail) before the first postinjection temperature measurement. For the combined administration, MDMA, COCA, or AMPH were dissolved directly in the 20% EtOH solution.

For experiment 1, the dose of EtOH was 1.5 g/kg, MDMA 6.6 mg/kg, COCA 10 mg/kg, and D-AMPH 1 mg/kg. The doses of AMPH, COCA, and MDMA were selected because preliminary experiments carried out in our laboratory showed that at 60 min after injection, we observed an almost equivalent hyperthermia among the three, but observed no stereotypy. The dose of MDMA, however, showed greater locomotor activation than the doses of either COCA or AMPH. A diagram of the overall treatment regimen is shown in Fig. 1. The administration regimen was chosen on the basis of our most recent experiments (e.g., Ben Hamida et al. 2007), in which we found this regimen to (1) minimize the risk for MDMA-induced lethality under normal ambient temperature conditions, (2) prevent desensitization of the ethanol-induced attenuation of the hyperthermia due to MDMA (as shown in Cassel et al. 2004), and (3) have no neurotoxic effects on serotonergic neurons at the same dose as herein.

For experiment 2, MDMA, AMPH, COCA, and EtOH preparations were identical to those used in our first experiment, except that the MDMA dilution was made such as to administer a dose of 3.3 mg/kg in 7.5 ml (i.p.), that of COCA as to administer 20 mg/kg, and that of AMPH as to administer 2 mg/kg. Ethanol, in a 20% *w*/*v* solution, was administered at the dose of 1.5 mg/kg either alone (as one of the controls) or in combination with AMPH, COCA, or MDMA. As this second experiment was run in two steps (AMPH and COCA in one, MDMA in the other), each step had its own controls (NaCl, EtOH).

In adult Long–Evans male rats, an i.p. dose of 1.5 g/kg EtOH typically results in a blood concentration of about 270 mg/dl, when measured 15 min postinjection (Ben Hamida et al. 2007), with a zero-order disappearance rate of 67 mg/dl h^{-1}. MDMA (and therefore also both other psychostimulant drugs) was administered together with EtOH to reduce the number of injections. Multiple injections may be stressful, and stress likely interacts with the effects of MDMA (Johnson et al. 2004).

Locomotor activity in the home cage

Activity measures for the rats were obtained in their home cages by automated recording devices, and all rats were tested at once as described previously (Cassel et al. 2004). The animals were left undisturbed during recording. Activity was first monitored on the first and last days of acclimation to the test room, as indicated in Fig. 1 (days −3 and −1). Then, over three additional days, all rats were acclimated to being injected i.p. with 0.9% NaCl at 12:00 noon. Activity was recorded during 2 h before and 4 h after each injection. Subsequently, on days 4, 6, 11, and 13, activity was recorded 2 h before and 6 h after injections of MDMA, COCA, or AMPH, in combination or not with EtOH, as recently described (Ben Hamida et al. 2007). Control groups were given NaCl or EtOH alone. The ambient temperature of the room during testing was 23±1°C.

Body temperature

For both experiments, rectal temperature was measured using a portable digital MultiLogger® thermometer (CHY 502, Bioseb, France) with a 0.1°C accuracy and an approximately 15 s measurement time. The probe was lubricated with medical petroleum jelly. Body temperature was measured 60 min before injection and, after injection,

at 1-h intervals over four consecutive hours. The ambient temperature during measurement of body temperatures was 23±1°C.

In the second experiment, just before recording the effects of MDMA and MDMA + EtOH vs controls, one thermometer failed, and we had to substitute another Device: Pic indolor Vedo Flex, Artsana-Grandate, Italy, accurate to 0.1°C, which was the one used in our first experiments (Cassel et al. 2004, 2005).

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) followed, where appropriate, by multiple comparisons using the Newman–Keuls test (Winer 1971). The analyses were made separately for each drug, using the corresponding controls. For analyses of temperature changes in experiment 1, we used a drug (NaCl, EtOH, psychostimulant, EtOH + psychostimulant), *X* day (1, 2, 3, 4) *X* h (−1, 1, 2, 3, 4) between-within subjects factors design. For analyses of activity changes, we used a drug (NaCl, EtOH, psychostimulant, EtOH + psychostimulant), *X* day (4, 6, 11, 13), *X* h (−1, 1, 2, 3, 4, 5, 6) mixed between-within subjects factors design. In experiment 2, the drug-induced effects were assessed only on two occasions, and the design of the ANOVA was adapted accordingly (the first within-subject factor, namely day, having only two levels instead of four).

Results of experiment 1

Locomotor activity in the home cage

Activity levels recorded during acclimation (see Fig. 1, days −3 to −1) were comparable across treatment groups (not illustrated). After saline injections (see Fig. 1; days 1 to 3), we observed a slight increase in locomotor activity in the first postinjection hour (as subsequently found on drug challenge days in controls; for illustration, see Fig. 2, first postinjection hour; notice the *Y*-scale change for MDMA data). Nevertheless, activity did not differ significantly among groups (not illustrated). Moreover, on drug challenge days, pre-injection activity among the groups showed no differences (as shown in Fig. 2).

Amphetamine

The results are shown in Fig. 2 (left). ANOVA of the activity scores, including the last pre-injection hour, showed a significant drug effect (F 3/37=1.6, $p<0.001$). Overall, AMPH increased locomotor activity compared to saline or EtOH ($p<0.05$, at most); EtOH attenuated this increase ($p<0.01$). There was also a significant day effect (F 3/111=6.5, $p<0.001$) and a significant day × drug interaction (F 9/111=6.4, $p<0.001$). The day effect was due to overall activity scores that were significantly higher on day 11 than on day 6 ($p<0.05$) and on day 13 than on days 4 and 6 ($p<0.01$). The day × drug interaction thus suggests sensitization.

In summary, AMPH increased locomotor activity, with clear-cut evidence for sensitization over repeated treatment administrations. When EtOH was coadministered with AMPH, there was neither increase in locomotion nor sensitization.

Cocaine

The results are shown in Fig. 2 (middle). ANOVA of the activity scores, including the last pre-injection hour, showed a significant drug effect (F 3/35=6.9, $p<0.001$), reflecting an overall increase in activity in COCA rats, compared to any of the other treatment groups ($p<0.05$, at least), these being not significantly different from each other. There was no significant day effect (F 3/105=2.0, $p<0.20$), but the day × drug interaction was significant (F 9/105=3.1, $p<0.01$). This interaction is reflected in increased locomotion in COCA-treated rats (day 13 vs 11, 6, or 4, $p<0.01$). ANOVA also showed a significant overall hour effect (F 6/210=32.8, $p<0.001$), and all interactions involving the hour factor, except day × hour (F 18/630=1.3), were significant, whether of the second (hour × drug: F 18/210=4.1, $p<0.001$) or of the third order (day × hour × drug: F 54/630=2.9, $p<0.001$). Conversely, in COCA + EtOH rats, the activity, which was initially high, evinced a decrease that was significant between days 11 or 13 and 4 ($p<0.001$) or 6 ($p<0.001$). The difference between days 4 and 6 or days 11 and 13 was not significant in this treatment group.

The overall hour effect was due to significantly larger overall activity scores during both hours that followed the injection compared to any of the five other hours (i.e., the pre-injection and the last four postinjection hours; $p<0.05$, at least), during which the activity scores did not differ significantly from each other. The hour × drug interaction was mainly due to the fact that the injections of COCA resulted in a significant activity increase in all groups, but the scores during the first postinjection hours in COCA and COCA + EtOH rats were significantly larger than in both other groups ($p<0.001$). The day × hour × drug interaction was mainly due to the fact that on the first, and for part on the second postinjection hours, the activity in COCA rats underwent an increase from injection to injection, which was significant between the day of the first drug injection (i.e., day 4) and any of the other injection days ($p<0.001$), and between the 6th and the 13th injection days ($p<0.05$).

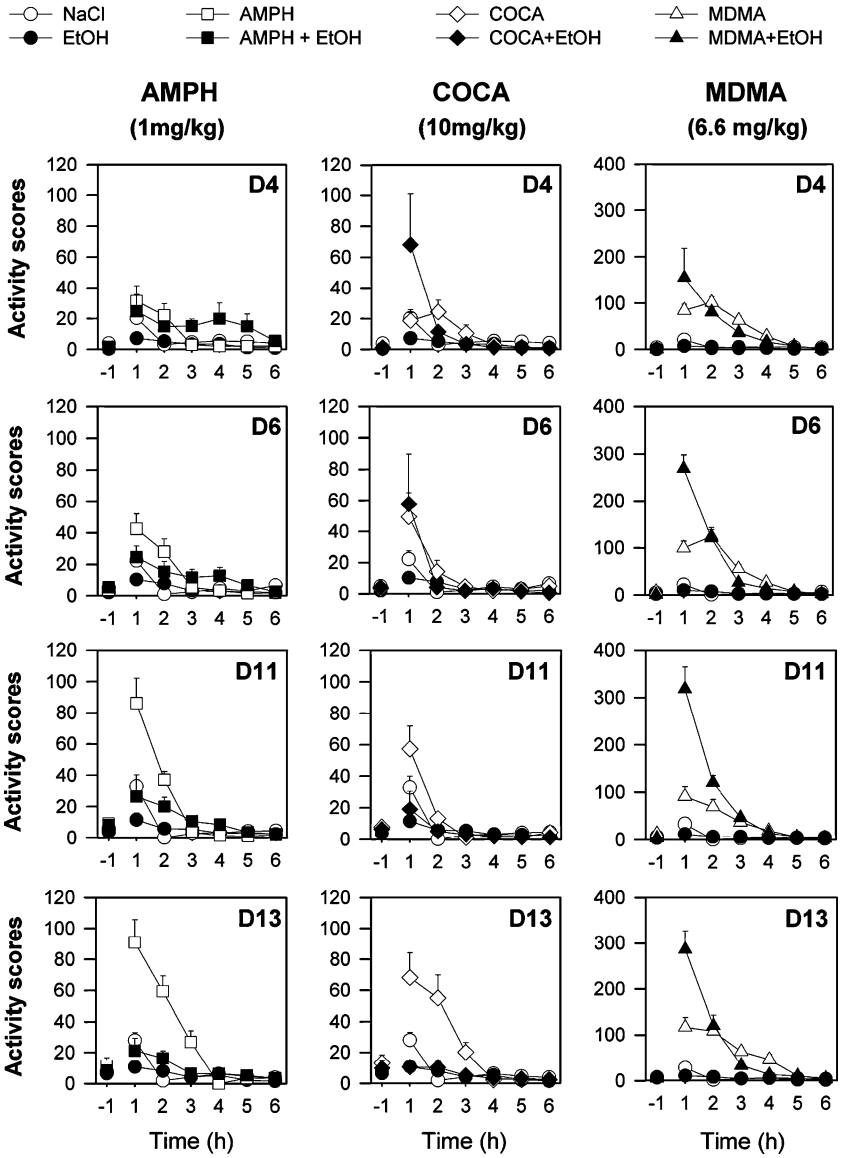

Fig. 2 Average (+SEM) locomotor activity scores recorded on days 4 (D4), 6 (D6), 11 (D11), and 13 (D13) during 1 h preceding (−1) and 6 h after (1–6) the injection of saline (NaCl), ethanol (EtOH), amphetamine either without (AMPH) or with ethanol (AMPH + EtOH), cocaine either without (COCA) or with ethanol (COCA + EtOH), and MDMA either without (MDMA) or with ethanol (MDMA + EtOH). Note that the *Y*-scale for the activity after MDMA treatment (*graphs on the right*) is larger than that used for AMPH or COCA. Doses were 1.5 g/kg for ethanol, 1 mg/kg for AMPH, 10 mg/kg for COCA, and 6.6 mg/kg for MDMA. All injections were made i.p. at a volume of 7.5 ml/kg. For a summary of all data, see Table 1

In summary, COCA induced a small increase in locomotor activity after the first injection with clear-cut evidence for sensitization over repeated treatments. When EtOH was coadministered with COCA, the initial increase in locomotor activity was larger than after COCA alone, but it subsequently decreased until a stage of no locomotor increase, as seen after the third and fourth injections.

MDMA

These results are shown in Fig. 2 (right). ANOVA of the activity scores showed a significant drug effect (F 3/34=92.7, $p<0.001$), with activity being significantly augmented in MDMA and MDMA + EtOH compared to NaCl or EtOH ($p<0.001$), and in MDMA + EtOH-treated rats compared to the MDMA-only ($p<0.001$). There was also a significant day effect (F 3/102=3.2, $p<0.05$) and a significant day × drug interaction (F 9/102=2.5, $p<0.05$). The day effect was due to overall activity scores that were significantly higher on days 11 and 13 than on day 4 ($p<0.05$). The day × drug interaction reflects increased locomotion in MDMA + EtOH-treated rats, an effect that stands out prominently from the three other groups on days 6, 11, and 13 ($p<0.001$ for all). ANOVA also showed a significant overall hour effect (F 6/204=155.2, $p<0.001$), and all interactions involving the hour factor were significant, whether of the second (hour × drug: F 18/204=58.9, $p<0.001$; day × hour: F 18/612=3.3, $p<0.001$) or of the third order (day × hour × drug: F 54/612=2.0, $p<0.001$). The overall hour effect was due to significantly larger overall activity scores during the three first hours that followed the injection compared to any of the four other hours (i.e., the pre-injection and the last three postinjection hours; $p<0.001$), to scores that were significantly higher

during the first compared to the second postinjection hour ($p<0.001$), and second compared to the third hour ($p<0.001$). The hour × drug interaction reflects that the scores during the first postinjection hour in MDMA + EtOH rats were significantly larger than in any other group ($p<0.001$), and they were also larger in MDMA rats compared to those receiving NaCl- or EtOH- ($p<0.01$). The day × hour interaction mostly reflected the increase in locomotor activity seen in MDMA or MDMA + EtOH rats, as the overall levels of activity recorded during the first postinjection hour on day 4 were significantly lower than those found on days 6, 11, and 13 ($p<0.001$). This difference was, in fact, mainly due to sensitization of the locomotor response in MDMA + EtOH rats, which also explains the day × hour × drug interaction. Indeed, on the first postinjection hour, the activity in MDMA + EtOH rats was significantly different between days 4 and 6 ($p<0.001$), between days 6 and 11 ($p<0.001$), and between days 11 and 13 ($p<0.05$), but not between days 6 and 13. Conversely, in the MDMA rats, the activity scores did not significantly differ from each other over drug challenge days.

In summary, MDMA produced an increase in locomotor activity, without apparent sensitization over treatment repetition. When EtOH was coadministered with MDMA, we noticed a potentiation of the effects of MDMA and a large sensitization across treatment days.

Body temperature

All data are shown as relative temperature changes expressed in ±°C from the basal temperature measured 1 h before each of the four injections. Basal temperatures during the hour before treatment ranged between 36.7 to 37.7°C with no consistent differences among the groups.

Amphetamine

The results are shown in Fig. 3 (left). ANOVA of the temperature changes showed a significant overall drug effect (F 3/24=25.2, $p<0.001$), which reflects an overall increase in body temperature after AMPH treatment vs NaCl ($p<0.001$), EtOH ($p<0.001$), or AMPH + EtOH ($p<0.001$). There was no effect of NaCl or EtOH. AMPH + EtOH produced marked decreases in body temperature compared to control ($p<0.01$). There was also a significant overall day effect (F 3/72=5.8, $p<0.01$) and a significant day × drug interaction (F 9/72=2.7, $p<0.01$). The day × drug interaction was due to a temperature increase in the AMPH condition on the second and subsequent treatments, whereas in AMPH + EtOH, the effect was a decrease in body temperature across all days. The significant drug × hour interaction (F 12/96=3.8, $p<0.001$) reflects the opposite direction of within session change in temperature observed between AMPH and AMPH + EtOH.

In summary, on each of the four injection days, AMPH induced hyperthermia, and on all days; the addition of EtOH to AMPH resulted in significant hypothermia.

Cocaine

We observed a significant main effect for drug COCA (F 3/24=20.2, $p<0.001$, Fig. 3 middle). COCA elevated body temperature on all treatment days compared to all other treatment conditions, ($p<0.001$ for all). A significant day × drug interaction (F 9/72=2.3, $p<0.05$) reflected a relatively stable temperature increase over days in the COCA treatment, whereas in COCA + EtOH rats, the hypothermia observed initially was not observed on subsequent treatment days. The significant drug × hour interaction (F 12/96=3.8, $p<0.001$) was due to the COCA-induced hyperthermia after the injection and to the transient hypothermia found in COCA + EtOH compared to NaCl or EtOH.

In summary, on each of the four injection days, COCA induced hyperthermia, but COCA + EtOH resulted in hypothermia only after the first two injections only.

MDMA

The results are shown in Fig. 3 (right). We observed a significant drug effect (F 3/24=6.7, $p<0.01$), which was due to temperatures that were significantly increased in MDMA rats compared to all other groups ($p<0.05$). There was also a significant overall day effect (F 3/72=6.5, $p<0.001$) and a significant day × drug interaction (F 9/72=2.3, $p<0.05$). The significant day effect was due to an overall increase of temperatures that was significantly greater on days 4 and 6, compared to days 11 and 13 ($p<0.05$). The day × drug interaction was due to a relatively constant temperature increase over days in MDMA, whereas in MDMA + EtOH, the increase that we observed lessened across treatments.

In summary, on each of the four injection days, MDMA produced hyperthermia. Addition of EtOH resulted in attenuation of the MDMA-induced hyperthermia. This attenuation progressed at each treatment so that by the fourth treatment, MDMA-related hyperthermia was completely absent. In no case did combined MDMA and EtOH produce hypothermia.

We never observed hypothermia when MDMA was given in combination with EtOH, whereas hypothermia occurred as a result of AMPH–EtOH or COCA–EtOH administrations. Therefore, we designed a second experiment in which EtOH was combined with half of the dose of MDMA used in experiment 1 (i.e., 3.3 mg/kg, i.p.).

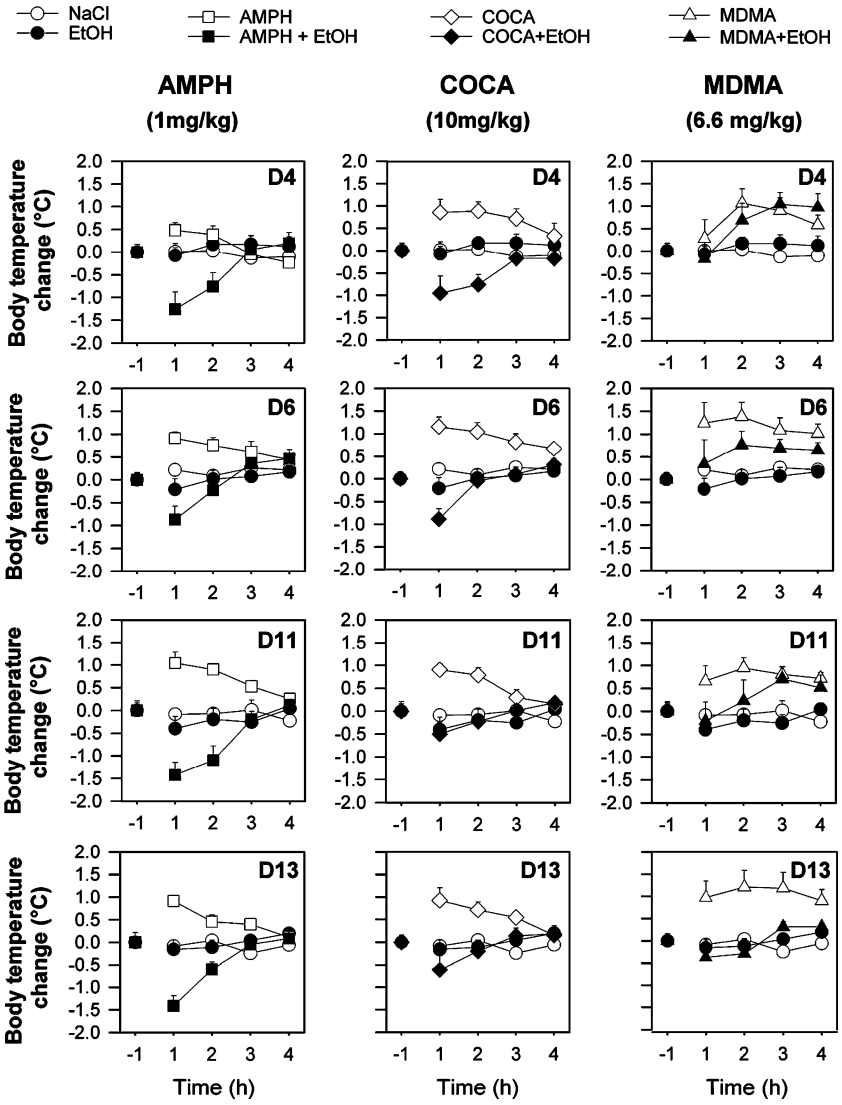

Fig. 3 Average (+SEM) body temperature changes recorded on days 4 (D4), 6 (D6), 11 (D11), and 13 (D13) right before (−1) and over 4 h after (1–4) the injection of saline (NaCl), ethanol (EtOH), amphetamine without (AMPH) or with ethanol (AMPH + EtOH), cocaine without (COCA) or with ethanol (COCA + EtOH), and MDMA without (MDMA) or with ethanol (MDMA + EtOH). Doses were 1.5 g/kg for ethanol, 1 mg/kg for AMPH, 10 mg/kg for COCA, and 6.6 mg/kg for MDMA. The *Y*-scale is the same for all drug conditions. All injections were made i.p. at a volume of 7.5 ml/kg. For initial body temperatures in °C on each day, see Results of experiment 1 and Results of experiment 2 sections. For a summary of all data, see Table 1

Results of experiment 2

Locomotor activity in the home cage

With the exception of the dose of AMPH, COCA, or MDMA and also the number of drug challenge days (two instead of four), the methods were exactly the same as in the first experiment. As in experiment 1, activity levels recorded during acclimation (see Fig. 1, days −3 to −1) were comparable across treatment groups (not illustrated). After saline injections (see Fig. 1; days 1 to 3), we observed a slight increase in locomotor activity in the first postinjection hour (as subsequently found on both drug challenge days in controls). Nevertheless, activity did not differ significantly among groups (not illustrated). On drug challenge days, pre-injection activity among the groups showed no differences (as shown in Fig. 4).

Amphetamine

The results are shown in Fig. 4 (left). ANOVA of the activity scores, including the last pre-injection hour, showed a significant drug effect (F 3/36=18.79, $p<0.001$). Overall, AMPH increased locomotor activity compared to saline or EtOH ($p<0.05$, at most); EtOH attenuated this increase ($p<0.01$). ANOVA also showed a significant overall hour effect (F 4/144=39.57, $p<0.001$) and a significant hour × drug interaction effect (F 12/144=11.14, $p<0.001$). The hour × drug interaction was due to a significant increase of locomotor activity in AMPH rats during postinjection hours 1 and 2 compared to NaCl, EtOH, or AMP + EtOH ($p<0.05$, for all). In addition, AMPH + EtOH rats were more active than NaCl or EtOH rats during the first hour postinjection ($p<0.05$). The drug × day × hour effect only tended to be significant ($p=0.07$); all other interactions were not significant.

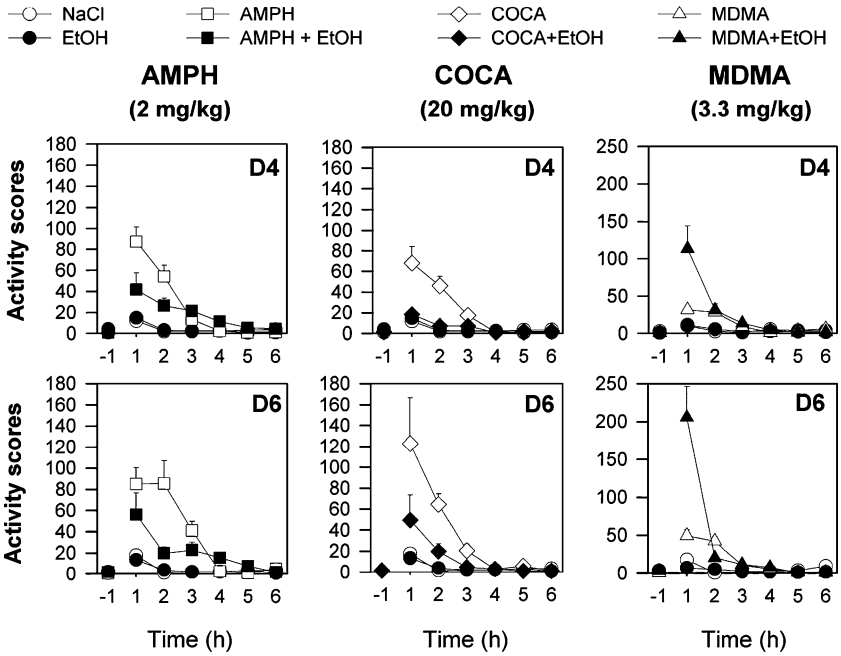

Fig. 4 Average (+SEM) locomotor activity scores recorded on days 4 (D4) and 6 (D6) during 6 h after (1–6) the injection of saline (NaCl), ethanol (EtOH, 1.5 g/kg), and AMPH (2 mg/kg), COCA (20 mg/kg), or MDMA (3.3 mg/kg) either without or with ethanol (+EtOH). All injections were made i.p. at a volume of 7.5 ml/kg. For initial activity scores, see Results of experiment 1 and Results of experiment 2 sections

In summary, AMPH increased locomotor activity and EtOH attenuated this effect.

Cocaine

The results are shown in Fig. 4 (middle). ANOVA of the activity scores, including the last pre-injection hour, showed a significant drug effect (F 3/36=17.33, $p<0.001$), which was due to the significant increase of activity in COCA rats compared to any of the other treatment groups ($p<0.001$, at least). In the latter groups, the activity scores did not differ from each other. There was a significant day effect (F 1/36=5.02, $p<0.05$), but the day × drug interaction was not significant (F 3/36=1.94, n.s.). The interactions of the second order involving the hour factor were significant, (hour × drug: F 12/144=6.90, $p<0.001$ and also day × hour: F 4/144=2.92, $p<0.05$). The hour × drug interaction was mainly due to the fact that the injections of COCA affected the activity during the two first postinjection hours or only during the first one when EtOH was given in addition ($p<0.05$).

In summary, COCA-treated rats were hyperactive during the two first hours that followed the injection, and ethanol prevented (day 4) or attenuated (day 6) this hyperactivity.

MDMA

The results are shown in Fig. 4 (right). ANOVA showed a significant drug effect (F 3/38=17.2, $p<0.001$). Activity was significantly increased in MDMA and MDMA + EtOH rats compared to NaCl or EtOH ($p<0.05$) and also in MDMA + EtOH rats compared to the MDMA only ones ($p<0.001$). There was also a significant day effect (F 1/38=14.4, $p<0.001$) and a significant day × drug interaction (F 3/38=6.5, $p<0.01$). The day effect was due to overall activity scores that were significantly higher on day 6 than on day 4 ($p<0.001$). The day × drug interaction reflected an overall significant sensitization in MDMA + EtOH-treated vs MDMA-only rats (D4 vs D6: $p<0.001$). ANOVA also showed a significant overall hour effect (F 5/190=23.3, $p<0.001$), and all interactions involving the hour factor were significant, whether of the second (hour × drug: F 12/152=11.9, $p<0.001$; day × hour: F 4/152=18.9, $p<0.001$) or of the third order (day × hour × drug: F 12/152=13.9, $p<0.001$). The hour effect was due to significantly larger overall activity scores during the first hour after the injection compared to any of the five other hours ($p<0.001$). The hour × drug interaction was mainly due to the fact that the scores during the first postinjection hour in MDMA + EtOH rats were dramatically larger than in any other group ($p<0.001$). The day × hour interaction mostly reflected the sensitization of the overall locomotor response found in MDMA + EtOH rats, as the overall levels of activity recorded during the first postinjection hour on day 4 were significantly lower than those found on day 6 ($p<0.001$). This difference also explains the day × hour × drug interaction.

In summary, MDMA produced an increase in locomotor activity, which did not sensitize with repetition of the treatment. When EtOH was coadministered with MDMA, there was a strong potentiation of the MDMA effects during the first hour and a large sensitization across both treatment days.

Body temperature

The data are shown as relative temperature changes expressed in ±°C from the basal temperature measured 1 h before each injection. Basal temperatures ranged between 36.7 and 37.7°C, with no consistent differences among the groups.

Amphetamine

The results are shown in Fig. 5 (left). ANOVA of the temperature changes showed a significant overall drug effect (F 3/25=10.21, p<0.001), which reflected an overall increase in body temperature after AMPH treatment vs NaCl (p<0.05), EtOH (p<0.001), or AMPH + EtOH (p<0.001). There were no significant differences between NaCl or EtOH or AMPH + EtOH. There was also a significant overall hour effect (F 4/100=5.43, p<0.01), and a significant hour × drug interaction (F 12/100=7.01, p<0.01). The hour × drug interaction was due to a temperature increase in AMPH compared to NaCl or EtOH and AMPH + EtOH rats. The responses on days 4 and 6 did not differ significantly from each other.

In summary, only AMPH induced a significant increase of body temperature, which was prevented by the addition of EtOH.

Cocaine

Data are shown in Fig. 5 (middle). ANOVA showed a significant main effect for drug (F 3/25=22.99, p<0.001), which reflected an overall increase in body temperature after COCA treatment vs NaCl (p<0.001), EtOH (p<0.001), or COCA + EtOH (p<0.001). There was no significant difference among NaCl and EtOH rats. In COCA + EtOH rats, the temperature was significantly weaker than in NaCl (p<0.01) and EtOH (p<0.05) rats. We also noticed a significant hour effect (F 4/100=4.08, p<0.05) and a significant hour × drug interaction (F 12/100=10.43, p<0.01).

In summary, on each of both injection days, COCA induced hyperthermia, but the addition of EtOH produced transient hypothermia.

MDMA

The data are shown in Fig. 5 (right). With exception of the dose and the temperature recording device (see Materials and methods section), the methods were the same as in the first experiment. One hour before injection, the mean temperature in all groups ranged between 37.0 and 37.4°C. After treatment, we observed a significant overall drug effect (F 3/36=5.8, p<0.01), which was due to temperatures that were significantly increased in MDMA rats compared to all other groups (p<0.01); there were no significant differences among the other three groups. There was also a significant overall day effect (F 1/36=6.6, p<0.05), but no significant day × drug interaction (F 3/36=1.1, n.s.). The day effect was due to an overall increase in temperatures that was significantly higher on day 6 compared to day 4. The significant hour effect (F 3/108=4.8, p<0.01) was due to overall temperatures which peaked on hours 2 and 3 compared to 1 (p<0.01). A significant hour × drug interaction was also found (F 12/144=7.9, p<0.001), due

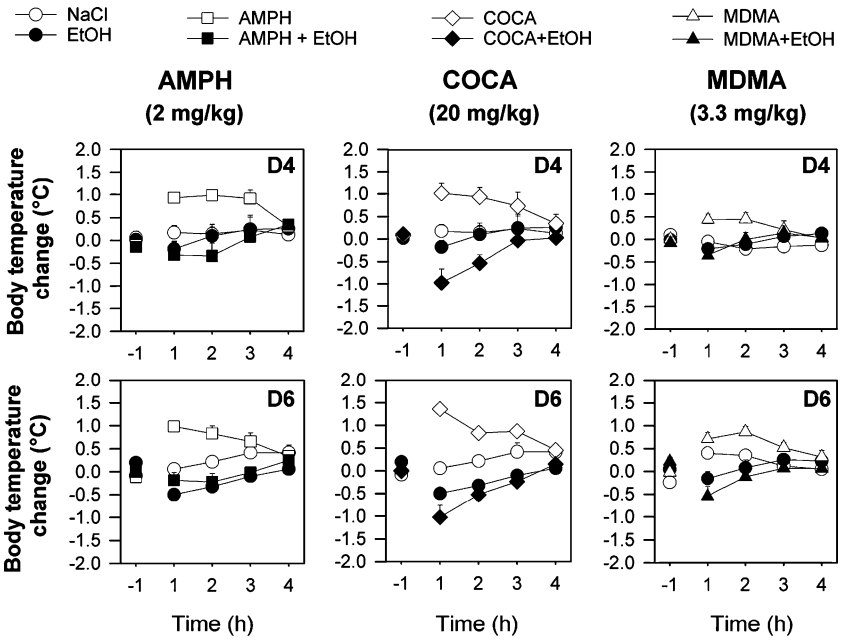

Fig. 5 Average (+SEM) body temperature changes recorded on days 4 (D4) and 6 (D6) over 4 h after (1–4) the injection of saline (NaCl), ethanol (EtOH, 1.5 g/kg), and AMPH (2 mg/kg), COCA (20 mg/kg), or MDMA (3.3 mg/kg) without (MDMA) or with ethanol (+EtOH). All injections were made i.p. at a volume of 7.5 ml/kg

to postinjection differences among temperature changes that vanished over time. To refine the analysis, data from D4 and D6 were analyzed separately from each other. On each of both days, there was a significant hour × drug interaction (D4: F 12/144=3.5; D6 F 12/144=7.1, $p<0.001$ in each case). On D4, the temperatures were significantly higher in MDMA rats compared to MDMA + EtOH rats ($p<0.01$). On D6, after one postinjection hour, the average temperature changes were significantly higher in MDMA rats compared to MDMA + EtOH rats ($p<0.001$), in which significant hypothermia was now observed ($p<0.05$).

In summary, on each of both injection days, MDMA produced hyperthermia, which was obviously weaker than at the dose of 6.6 mg/kg. Addition of EtOH resulted in attenuation of the MDMA-induced hyperthermia, even resulting in significant hypothermia on D6.

Discussion

The impetus of the present study was our previous findings on MDMA + EtOH interactions with respect to locomotor activity and thermoregulation (Ben Hamida et al. 2006, 2007; Cassel et al. 2004, 2005). In rats, not necessarily in all other species (Easton and Marsden 2006), hyperactivity and hyperthermia are two acute manifestations of MDMA intoxication (Green et al. 2003). The hyperthermia is a pharmacological effect rather than a consequence of hyperactivity (Crean et al. 2006; Dafters 1994; Green et al. 2004a).

The results of experiment 1 (Table 1) confirmed that AMPH and COCA, like MDMA under normal ambient temperature conditions, induce hyperthermia (Ansah et al. 1996; Gonzalez 1993; Lomax and Daniel 1990; Green et al. 2003). It is noteworthy that the doses used in experiment 1 were determined a priori to produce an initial hyperthermia of approximately the same amplitude for all three psychostimulants. When EtOH was coadministered with AMPH or COCA, we observed severe hypothermia, whereas in combination with MDMA (6.6 mg/kg), EtOH only attenuated the MDMA-induced hyperthermia. In addition, the magnitude of the latter effect increased with repeated injections, suggesting sensitization, so that by the final injection, we observed complete prevention of hyperthermia. In contrast, the thermoregulatory effects of AMPH + EtOH were relatively consistent over days, and the initial hypothermia in the COCA–EtOH group diminished progressively, suggesting tolerance. The results also confirmed the well-established observation that AMPH, COCA, and MDMA induce locomotor hyperactivity. This hyperlocomotion underwent sensitization in both AMPH- and COCA-treated rats, as already documented. EtOH blocked the effects of AMPH on activity, transiently potentiated the locomotor effects of COCA, and dramatically potentiated MDMA hyperactivity, an effect undergoing sensitization.

In the second experiment, at a lower dose of MDMA, which now induced a hyperactivity that was relatively comparable to that found after AMPH or COCA in experiment 1, we confirmed both the EtOH-induced potentiation of MDMA hyperlocomotion and the sensitization of this response. We also showed that MDMA + EtOH now resulted in hypothermia, as with AMPH– and COCA–EtOH combinations in the first experiment. Concerning AMPH and COCA, the results were relatively comparable to those found in experiment 1 on hyperthermia. On locomotor activity, the levels were higher after AMPH or COCA alone, but hyperactivity was still attenuated (not potentiated) by EtOH. Altogether, these findings point towards both similarities and differences between the effects of the three drugs, especially when associated to

Table 1 Summary of the main findings

Drugs	Initial change of activity	Effects of injection repetition	Initial change of temperature	Effects of injection repetition
AMPH (1 mg/kg)	Hyperactivity	↑	Hyperthermia	↑
AMPH + EtOH (1 mg/kg+1.5 g/kg)	No change	→[a]	Hypothermia	→
COCA (10 mg/kg)	Hyperactivity	↑	Hyperthermia	→
COCA + EtOH (10 mg/kg+1.5 g/kg)	Larger hyperactivity[b]	↓	Hypothermia	↓
MDMA (6.6 mg/kg)	Hyperactivity	→	Hyperthermia	↑
MDMA + EtOH (6.6 mg/kg+1.5 g/kg)	Larger hyperactivity[c]	↑	No hypothermia[d]	↑

Note that EtOH alone induced no significant modification of body temperatures (compared to NaCl), but reduced the activity levels.
→ No modification of the response, ↑ significant sensitization of the response, ↓ significant desensitization of the response, *AMPH* D-amphetamine, *COCA* cocaine, *EtOH* ethanol, *MDMA* 3,4-methylenedioxymethamphetamine.
[a] Meaning that sensitization towards amphetamine might be prevented by EtOH.
[b] Only after the initial injection, then EtOH prevented cocaine-induced hyperactivity.
[c] Enormous increase of hyperactivity in the presence of EtOH and the potentiating effect even gained in magnitude with repeated injections.
[d] Weak attenuation after the initial injection, but then EtOH progressively reversed the hyperthermia with injection repetition.

EtOH. They also indicate that a full investigation based on a larger dose–response approach and complementary investigation techniques (e.g., in vivo microdialysis) is worth the effort.

Concerns about the serotonergic toxicity of our MDMA ± EtOH regimen

MDMA preferentially causes increased serotonin release. Depending on the dose or/and treatment regimen, however, it may induce long-lasting serotonergic toxicity, which is detectable 6–7 days after drug administration (Colado et al. 1998; O'shea et al. 1998) and may be enhanced by prior heavy EtOH intoxication (Izco et al. 2007; see also Jones and Cassel 2007). Furthermore, MDMA-related toxicity may affect thermoregulatory (Saadat et al. 2005; Marston et al. 1999) and behavioral (Marston et al. 1999; Brennan and Schenk 2006) responses to subsequent MDMA exposure. Because our treatment regimen extended over a 10-day period, it is critical to show that the dose/regimen of MDMA used in the current experiments did not produce serotonergic toxicity. As shown elsewhere (Ben Hamida et al. 2007), using the same administration regimen and the highest of both doses of MDMA as herein, we observed no evidence for serotonergic toxicity by any of the treatments.

Concerns about pharmacokinetics vs pharmacodynamics

At the present stage of our knowledge, we cannot propose precise mechanisms underlying the differential physiological responses to the three EtOH–drug associations. Furthermore, a larger dose–response could be necessary. Reasons to the differences reported herein may be multiple and include both pharmacokinetic and pharmacodynamic peculiarities of each drug association, such as (1) the drug-specific formation of different and more or less physiologically active metabolites when coadministered with EtOH; in turn, depending on the drug, these metabolites might then intensify, prolong, counterbalance, or leave unchanged the effects of the original compound [e.g., cocaethylene in case of COCA + EtOH; (Pan and Hedaya 1999a, b)], (2) increased/decreased availability in the plasma or/and the brain of one of the drugs in the presence of EtOH, as shown for instance for COCA in the rat (Hedaya and Pan 1996, 1997), or MDMA in mice (levels increased in the brain; Johnson et al. 2004) or humans (levels increased in plasma; Hernandez-Lopez et al. 2002), (3) more or less pronounced additive or subtractive synergism between the effects of any of the three psychostimulants and EtOH on the release of monoamines, and particularly of dopamine and 5-HT, (4) interactions between the effects of the drug-induced increase of monoamine release and the effects of EtOH on various receptors involved in the modulation of this release (e.g., adenosine receptors, NMDA receptors, and even nicotinic receptors), and (5) more or less efficient EtOH-induced vasodilatation, which may lead to an increased heat dissipation that could partly (MDMA), completely (AMPH), or excessively (COCA) counterbalance the vasoconstrictor effects of MDMA, AMPH, and COCA, respectively. The latter mechanism might then lead to weaker or larger heat dissipation (although in the rat, such dissipation mainly occurs at the level of the tail; Green et al. 2005) and, thus, to more or less effective prevention of hyperthermia. For MDMA, for instance, it was shown that part of the thermoregulatory problems encountered by rats given neurotoxic regimens of MDMA resulted from their inability to lose heat by vasodilatation in the tail (Green et al. 2005). Thus, EtOH, due to its endothelium-mediated vasodilatation properties, might contribute to weaker hyperthermia in MDMA-treated rats by easing heat dissipation. Further studies are necessary to progress on this issue and to understand which among the aforementioned potential substrates may explain not only the thermoregulatory consequences of associating EtOH to AMPH, COCA or MDMA, but also both the similarities and peculiarities of each association, as beside some similarities on thermoregulation, clear-cut differences in the outcome of the three types of EtOH-drug associations were observed in terms of locomotor activity.

Psychostimulant and ethanol–psychostimulant drug combination effects on body temperature

MDMA-induced hyperthermia is thought to involve serotonergic and dopaminergic components, the latter of which probably predominates. Indeed, although hyperthermia was related to MDMA-induced increase of 5-HT release (Shankaran and Gudelsky 1999), more recent studies provided evidence in favor of a dopaminergic mediation of MDMA's thermoregulatory effect (Beveridge et al. 2004; Goni-Allo et al. 2006; Green et al. 2004b; Kankaanpaa et al. 1998; Mechan et al. 2002; Saadat et al. 2005). Noradrenergic mechanisms may also contribute to the hyperthermic effects of MDMA, namely, via α-adrenergic receptors (e.g.,(Bexis and Docherty 2005)), and there is evidence that activation of the $GABA_B$ receptor reverses this effect (Bexis et al. 2004).

Therefore, our observations of AMPH, COCA, and MDMA-induced hyperthermia, whatever the dose, are likely linked principally to alterations in dopamine release and/or reuptake, the common effect among the three stimulant drugs. This is further supported by previous studies showing the involvement of dopaminergic mechanisms in AMPH-induced hyperthermia (Ulus et al. 1975). In the case of MDMA, the effects observed might have been related to increased dopamine, whether directly or

indirectly, and perhaps even via increased 5-HT release (Alex and Pehek 2007). It is noteworthy that AMPH is a much less potent inhibitor of the serotonin transporter (SERT) than of the dopamine transporter (DAT) in mice and humans (Han and Gu 2006). While not absolute proof per se of dopamine mediation of hyperthermia, this does offer some support. AMPH has the highest affinity for the norepinephrine transporter (NET; (Han and Gu 2006)), but any involvement of norepinephrine in AMPH hyperthermia has been questioned (Kostowski et al. 1982). Preferential involvement of dopamine in COCA-induced hyperthermia is also reported (Callaway and Clark 1994). Nevertheless, despite arguments in favor of a dopamine mediation of the hyperthermia induced by each drug, it remains possible that the modulation of serotonin release, at least in the case of MDMA, has a role, perhaps via effects on heat dissipation (Saadat et al. 2005). Depending on the subtype of 5-HT receptors activated, serotonin has mainly stimulating and only limited inhibitory actions on dopamine release (see (Alex and Pehek 2007) for review).

COCA-induced hyperthermia confirms earlier reports (Ansah et al. 1996; Gonzalez 1993), and it is noteworthy that in the current study, the doses of 10 and 20 mg/kg induced almost comparable hyperthermia. In humans, this hyperthermia may result from impaired heat dissipation (Crandall et al. 2002). In vitro, COCA-induced inhibition of the SERT and DAT (and even of the NET) occurs in a very narrow concentration range (K_I difference less than twofold in mice and threefold in humans), but these close affinities for the transporters do not contradict the possibility of dopamine playing a major role in MDMA-related hyperthermia; in fact, the K_I of MDMA is similar to that of AMPH for the DAT. In the case of MDMA, again, it must be kept in mind that the effect on dopamine release might be indirect and involves a 5-HT link, whereas in the case of AMPH, it may be more direct, thus, involving a more focused action on the DAT.

AMPH + EtOH resulted in marked hypothermia for about 2 h after each of the four injections at the dose of 1 mg/kg. At the higher dose, namely, 2 mg/kg, EtOH did not induce marked hypothermia, but prevented hyperthermia. Initially, COCA + EtOH also induced hypothermia, but this response diminished with repeated injections at the lowest of both doses used (i.e., 10 mg/kg). MDMA + EtOH produced less hyperthermia than MDMA alone, and this effect increased with subsequent injections up to complete prevention in experiment 1. In our second experiment, there was EtOH-induced hypothermia in rats given 3.3 mg/kg MDMA, but only after the second injection (day 6). These results demonstrate clearly that the combination of EtOH with each of the psychostimulants, whose initial pyretic effects were of comparable magnitude, produces partly comparable, partly different patterns of effects.

Interestingly, EtOH increases the dopamine levels in the nucleus accumbens of COCA-treated rats (Lindholm et al. 2001) and interacts synergistically with COCA on dopaminergic neurons of the ventral tegmental area (Bunney et al. 2000). It also enhances the locomotor effects of COCA in mice (Masur et al. 1989). Similar effects were found with AMPH in rats (Duncan and Cook 1981). Unlike AMPH, synergistic effects of EtOH and COCA can partly be explained by the production of cocaethylene (Bunney et al. 2001; Pan and Hedaya 1999a, b, c). Thus, if dopamine is involved in the psychostimulant-induced hyperthermia, the fact that EtOH in combination with AMPH or COCA produced hypothermia can probably not be simply accounted for by an EtOH-induced modulation of dopamine effects. Following on the observations of our second experiment, it appears that EtOH and MDMA together can eventually produce hypothermia at lower doses of MDMA. This observation indicates that the pyretic effects of EtOH–psychostimulant combinations may primarily depend on the dose of these agents combined with an identical dose of ethanol. That hyperthermia induced by MDMA lasted longer than that resulting from AMPH or COCA is likely related to differences in the half-life of each drug (e.g., about 0.5 h for COCA and 2.2 h for MDMA). All these observations suggest that the mechanism of action of MDMA + EtOH could be partly different from, but also partly similar to that of COCA–EtOH or AMPH–EtOH. We did observe hypothermia with MDMA + EtOH at the lower dose. This suggests to us that the 5-HT-DA profile at each dose may be substantially different and warrants further investigation.

Psychostimulant and ethanol–psychostimulant drug combination effects on locomotor activity

Two properties common to AMPH, COCA, and MDMA are (1) an increase of synaptic dopamine, and (2) an increase of locomotor activity, presumably by augmenting dopamine concentrations in the mesolimbic and nigrostriatal terminal fields (Green et al. 2003; Gold et al. 1989). Because all these drugs also interact with the SERT [although much less for AMPH than for COCA and MDMA; (Han and Gu 2006)], a serotonergic contribution to increased locomotion, as proposed by others, is compatible with our current observations that in our first experiment, MDMA had a much stronger effect on locomotion than either AMPH or COCA (Geyer 1996; Rothman and Baumann 2003; Uhl and Lin 2003). This could simply be explained by a dose effect because after 2 mg/kg AMPH and 20 mg/kg COCA, we observed activity levels that were closer to those seen after 6.6 mg/kg MDMA. Because AMPH, COCA, and MDMA have high affinity for the NET, norepinephrine may also be involved (Vanderschuren et al. 2003).

MDMA-induced hyperactivity has been related to serotonin release (Green et al. 2003), as shown with various 5-HT agonists and/or antagonists, or 5-HT receptor mutants (Bankson and Cunningham 2002; Fletcher et al. 2002; Herin et al. 2005; Kehne et al. 1996; McCreary et al. 1999; Scearce-Levie et al. 1999). There is also evidence that dopamine receptors (D_1, D_2, and perhaps D_3) contribute to the hyperlocomotor effects of MDMA (Bubar et al. 2004; Risbrough et al. 2006). Finally, a contribution of alpha adrenoceptors is also possible (Bexis and Docherty 2006).

In part, these observations hold true for COCA, whose locomotor effects are attenuated by blockade of 5-HT_{2A} (Fletcher et al. 2002; McMahon and Cunningham 2001), D_1 (Kita et al. 1999; Steketee and Braswell 1997; O'Neill and Shaw 1999), and D_2 receptors (Kita et al. 1999), or by the activation of 5-HT_{2C} receptors (Fletcher et al. 2002, 2006). Systemic or region-targeted blockade of 5-HT_{1B} receptors reduces (Hoplight et al. 2005; Przegalinski et al. 2002), and their region-targeted activation (Przegalinski et al. 2002) or their viral transfection-induced overexpression in the ventral tegmental area (Neumaier et al. 2002), potentiates the locomotor effects of COCA. Finally, AMPH-induced hyperlocomotion is attenuated by a 5-HT_{2A} antagonist (Moser et al. 1996), or more or less, selective D_1 or D_2 receptor antagonists (Bast et al. 2002; Jackson et al. 1994). All these results provide evidence that AMPH, COCA, and MDMA have in common several pharmacological targets mediating their effects on hyperactivity.

Beside similarities among the effects of the three EtOH–drug combinations, our findings also produce evidence for unique action profiles on locomotor activity for each of them given repeatedly and in combination with EtOH. Above all, the striking sensitization produced by the combination of EtOH and MDMA, but not by MDMA alone, or by COCA + EtOH or AMPH + EtOH is a totally new finding. The dose–response curve of COCA is not bell-shaped, suggesting that a high dose of AMPH could induce effects more similar to those of MDMA, which we confirmed in experiment 2. For AMPH, however, the dose–response curve is bell-shaped (Antoniou et al. 1998), meaning that a higher dose could result in stereotypies, which are accompanied by decreased activity levels. The fact that these levels were further increased at the dose of 2 vs 1 mg/kg suggests that the high dose was still below the stereotypies threshold. Of course, the mechanisms underlying the different profiles become an important focus for enquiry. MDMA differs from COCA and AMPH in its higher affinity for the SERT vs DAT (about eight times larger vs 1.5 and 42 times weaker, respectively; Han and Gu 2006). Therefore, it is tempting to propose that the EtOH-induced potentiation of psychostimulant-induced hyperlocomotion might occur only when the serotonin/dopamine balance is largely in favor of serotonin, which is the case with MDMA, but neither with COCA nor with AMPH. If so, the potentiating effect of EtOH in MDMA-treated rats should be reduced in 5-HT depleted rats. Even at the higher doses of AMPH and COCA, we did not observe potentiation of the locomotor response by EtOH. With the lower dose of MDMA, which induced activity levels close to those found in our first experiment with AMPH or COCA, the EtOH-induced potentiation was still observed, suggesting that EtOH–MDMA combinations may have unique effects on activity (vs EtOH–AMPH or EtOH–COCA) and perhaps unique psychostimulant consequences.

Conclusions

Perhaps our most important finding is that EtOH interacts with MDMA in some ways that we would not have predicted, knowing initially how AMPH, COCA, and MDMA act on thermoregulation and locomotor activity. Although there were similarities among the effects of AMPH, COCA, and MDMA (hyperthermia and hyperactivity), the combination of these drugs with EtOH resulted in some differences in essentially their locomotor effects. We observed a progressive increase in the protection of MDMA-hyperthermia by EtOH. The sensitization to the effects of MDMA + EtOH on locomotor activity is a confirmation (Ben Hamida et al. 2007), but we show herein that it is unique to MDMA vs AMPH or COCA combined with EtOH. The interactions that we observed with COCA + EtOH and AMPH + EtOH were not nearly that dramatic as with MDMA + EtOH, although the latter might partly relate to dose as concerns thermoregulation. From a public health viewpoint, the fact that EtOH synergistically potentiated MDMA hyperlocomotion while protecting against MDMA hyperthermia could be regarded as an argument for people to use lower doses of MDMA and to "wash" the pills down with alcohol. This would be an incorrect conclusion because (1) all of our experiments have been carried out at laboratory temperatures of 21–23°C, whereas recreational use of MDMA often occurs in warmer environments (about 26°C; Cole et al. 2005); (2) in a recent experiment carried out at an ambient temperature of 32°C, we found the dose of 6.6 mg/kg MDMA with or without 1.5 g/kg EtOH to kill all treated rats within less than 2 h post-administration, and there was no evidence for EtOH-induced attenuation of the hyperthermia produced by MDMA (Cassel et al. 2007). Furthermore, all of our work on the combination of these drugs was in rats. Whether there is direct application to humans, thus, gives occasion for caution (see Easton and Marsden 2006). Finally, our present results should also be confirmed in rats given EtOH before MDMA, as most MDMA users seem to consume ethanol before they take ecstasy (Barrett et al. 2006).

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