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Ethanol–ecstasy (MDMA) interactions in rats: Preserved attenuation of hyperthermia and potentiation of hyperactivity by ethanol despite prior ethanol treatment

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Abstract

Recreational use of ecstasy, or (±)-3,4-methylenedioxymethamphetamine (MDMA), is often associated with other drugs, among which ethanol is one of the most common. Little is known, however, about the interaction between these two drugs. Using a daily ethanol and/or MDMA administration regimen, we recently showed that ethanol potentiated the hyperactivity (in the home cage), but attenuated the hyperthermia induced by MDMA. The prevention of hyperthermia occurred only on the first of four daily ethanol–MDMA treatments, indicating possible tolerance to ethanol. In order to test the tolerance hypothesis, we treated Long-Evans adult male rats with ethanol on 4 consecutive days prior to their first treatment with MDMA–ethanol. Our results first confirmed that ethanol (1.5 g/kg, i.p.) potentiates the psychomotor effects of MDMA (10 mg/kg, i.p.), while attenuating its pyretic effects (6.6 mg/kg, i.p.). The results also showed that both the potentiation of locomotor activity and the attenuation of hyperthermia by ethanol are not at all altered by prior ethanol treatment. This indicates that tolerance to ethanol per se does not account for what appears to be tolerance to the ethanol–MDMA combination, thus indicating that ethanol–MDMA combination likely has unique pharmacological effects.

Keywords: (±)-3,4-Methylenedioxymethamphetamine; Ecstasy; Ethanol; Locomotor activity; Rats; Temperature; Tolerance

1. Introduction

The amphetamine derivative (±)-3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a popular recreational drug used by young people, particularly in the 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 in the brain, psychostimulant effects, hyperthermia, and can be fatal (Schifano, 2004). In rats and primates (not mice) MDMA may also result in long-term depletion of hippocampal, striatal and cortical serotonergic markers (Cole and Sumnall, 2003; Green et al., 2003; Taffe et al., 2001). It is noteworthy, however, that the thermoregulatory and locomotor effects of MDMA may be different between rodents and primates, in which the pyretic response to MDMA appears less sensitive to ambient temperature, and no hyperlocomotion is observed (Taffe et al., 2006).

In humans, MDMA is frequently taken in combination with other drugs (Scholey et al., 2004). One of the drugs most frequently taken in combination with MDMA is ethanol (Lora-Tamayo et al., 2004; Pedersen and Skrondal, 1999; Schifano, 2004). Recently, we found that ethanol dramatically potentiated the hyperlocomotion induced by MDMA, but surprisingly, attenuated its hyperpyretic effects (Cassel et al., 2004, 2005). Although ethanol was found to potentiate the MDMA-induced hyperactivity on each of 4 consecutive days, this potentiation seemed to decline over the first 3 days (Cassel et al., 2004). More remarkably, the attenuation of MDMA hyperthermia by ethanol was found only on the first day,

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suggesting that subjects might develop tolerance to this effect of ethanol. Furthermore, although informative as to ethanol–MDMA interactions, none of these studies (Cassel et al., 2004, 2005) addressed the question about whether previous ethanol ingestion without co-administration of MDMA would attenuate the apparent protective effect of ethanol on MDMA hyperthermia. In Western countries, many young people are exposed to ethanol early in life, sometimes even repeatedly, which means that many may have developed some tolerance to ethanol when taking ecstasy for the first time and concomitantly with ethanol. In our recent studies, the rats were naïve to both drugs at the time of administration of either or in combination. Thus, it is possible that if our animals had received ethanol on one or more occasions prior to its being combined with MDMA, the results might have been different, *viz.*, no potentiation of locomotor activity and no initial protection against MDMA hyperthermia.

Consequently, in the present study, we investigated the effects of MDMA and ethanol treatments on both locomotion and body temperature in rats that had been treated with ethanol prior to MDMA–ethanol administration as compared to controls that were naïve to ethanol.

2. Materials and methods

2.1. Subjects

Eighty male Long-Evans rats (3 months old; Centre d'Elevage R. Janvier, Le Genest-St-Isle, France) were used. They were housed individually in transparent Makrolon cages ($42 \times 26 \times 15$ cm^3) under controlled temperature (23 °C) and a 12/12 h light/dark cycle (lights on at 7:00 a.m.). Food and water were provided *ad libitum*. After arrival, the animals were allowed to acclimate to the laboratory for 1 week, during which they were handled for 5 min daily by two experimenters. At the end of that week, they were randomly assigned to one of two experiments, one in which locomotor activity was assessed ($n=48$) and one in which body temperature was measured ($n=32$). 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, 86-23, revised 1985). All efforts were made to reduce the number of animals to its minimum regarding statistical constraints.

2.2. Pharmacological treatments

The ethanol solution (20% w/v) was prepared from absolute ethanol diluted in 0.9% NaCl. MDMA ((±)-3,4-methylenedioxymethamphetamine; NIDA, USA) was diluted in 0.9% NaCl. Both drugs, whether administered alone or in combination, as well as the NaCl solution, were injected intraperitoneally in a volume of 7.5 ml/kg, 1 to 5 min before activity recording was started, or 30 or 60 min (see below for detail) before the first post-injection temperature measurement. For the combined administration, MDMA was dissolved directly in the 20% ethanol solution. Ethanol was always given at a dose of 1.5 g/kg, whereas MDMA was injected at a dose of 10 mg/kg (locomotor activity) or 6.6 mg/kg (body temperature). Given i.p., in Long-Evans adult rats, a dose of 1.5 g/kg ethanol typically results in a blood concentration of about 230 mg/dl, when measured 15 min post-injection (personal unpublished data), with a zero-order disappearance rate of 67 mg/dl/h. MDMA was administered together with ethanol in order to reduce the number of injections. Injections may be stressful, and stress may interact with the effects of MDMA (e.g., Johnson et al., 2004). For the body temperature experiment, the dose of MDMA was reduced to 6.6 mg/kg because temperature recordings require frequent handling, which, due to stress, may potentiate the effects of MDMA (e.g., Johnson et al., 2004). Furthermore, in our previous experiments assessing the pyretic effects of MDMA in Long-Evans male rats, the dose of 10 mg/kg was occasionally lethal, which was not the case at 6.6 mg/kg, a dose sufficient to induce hyperthermia. In our hands, using the activity test described hereafter, this dose induces a five-fold increase (compared with saline) in spontaneous activity during the first hour after administration (unpublished preliminary data).

2.3. Locomotor activity measurements

Spontaneous activity of the rats was measured in their home cage and all rats were tested at once as in our former experiment (Cassel et al., 2004). No experimenter entered into the room in which activity was measured during recording. The cages were taken from the colony room and placed on shelves (8 cages/shelve) in a separate room (same light conditions as in the colony room). All rats had free access to food and water during activity recording. Each cage contained two crossing infrared light beams targeted on two photocells, 4.5 cm above floor level and 28 cm apart. The number of crossings in the cage (successive interruptions of the beams, and thus only 2-dimensional movements) was monitored continuously by a microcomputer. Activity was first monitored continuously during 2 days, starting the day on which rats were introduced to the testing conditions (Days −2 and −1, in Fig. 2). Then, over 3 days (Days 1, 2 and 3, in Fig. 2), rats were injected with a NaCl solution at 12:00 p.m. to familiarize them with the injection procedure. Activity was recorded during 2 h before and 2 h after each injection. Subsequently, on Days 4, 5, 6 and 7, rats were further injected with 0.9% NaCl or with ethanol (1.5 mg/kg, i.p.). On Day 8, half the rats treated with NaCl were given MDMA, and the other half were treated with a mixture of ethanol (1.5 mg/kg) and MDMA (10 mg/kg). The same was done for those that had received prior ethanol injections (see Fig. 1 for protocol summary). The ambient temperature during measurement of locomotor activity was 22±1 °C. On the challenge day, activity was recorded during 3 consecutive hours, a period sufficient to allow ethanol-induced potentiation to vanish.

2.4. Body temperature

Rectal temperature was measured with a Pic indolor Vedo Flex (Artsana-Grandate, Italy) digital thermometer with a 0.1 °C accuracy, and lubricated with medical petroleum jelly. Each

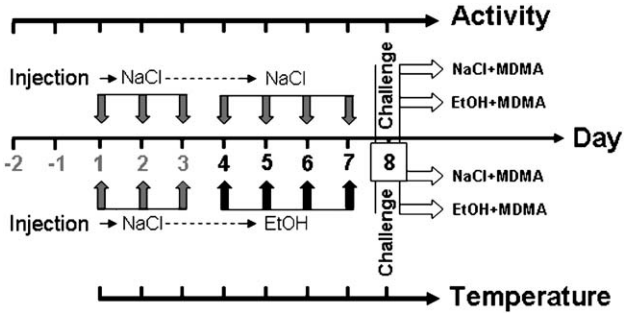

Fig. 1. Summary of the protocol used. Rats were first exposed to the test conditions (Days −2 and −1) and subsequently injected with saline (Days 1–3; NaCl 0.9%, 7.5 ml/kg, i.p.). On the next 4 days (Day 4 – Day 7), half the rats were injected with ethanol (20% w/v, 1.5 g/kg, i.p.), while the other half was injected with NaCl. On Day 8, half the rats pre-treated with NaCl and half the rats pre-treated with ethanol were given a MDMA (10 mg/kg, i.p.) challenge, the other halves being subjected to an Ethanol (1.5 g/kg)+MDMA (10 mg/kg) challenge.

measurement took approximately 30 s. All injections were made at about 12:00 p.m. All rats were first injected with NaCl, once per day during 3 consecutive days, as was done for activity measurements. Body temperature was measured once, 60 min before and 30 min after each injection. Subsequently, on Days 4, 5, 6 and 7, rats were again injected daily, with NaCl or with ethanol (1.5 mg/kg, i.p.). Temperatures were also taken 30 min after ethanol injection. On Day 8, half of the rats treated with NaCl or EtOH were injected with MDMA, and the other half were treated with a mixture of ethanol (1.5 mg/kg) and MDMA (6.6 mg/kg). On this challenge day, the temperature was measured 60, 120, 180 and 240 min after injection (see Fig. 1 for protocol summary). Throughout this study, the ambient temperature was 22±1 °C.

2.5. Statistical analysis

All data were analyzed using ANOVA or Student's *t*-test for paired samples. ANOVA was followed, where appropriate, by multiple comparisons using the Newman–Keuls test (Winer, 1971). Factors considered were Group (MDMA, EtOH+MDMA, on challenge day), Hour (i.e., 2, 3 or 4, depending on the experimental step), and Ethanol pre-exposure (pre-exposed or not).

3. Results

3.1. Effects of EtOH and MDMA on locomotor activity after prior ethanol experience

The data are shown in Fig. 2. ANOVA of the activity scores recorded during the first 2 h that followed the first exposure of the rats to the test conditions (no injection; Fig. 2, Day −2) showed no significant Group effect ($F(3,39)=1.4$) and no significant Group×Hour interaction ($F(3,39)<1.0$). The Hour effect was significant ($F(1,39)=71.7$, $p<0.001$), as shown by dramatic reduction of activity during the second as compared to the first hour. On the second day (no injection, Day −1), the overall activity scores were much lower than on the previous day, reflecting habituation of the rats to the test conditions. On the next three NaCl injection days (Day 1 – Day 3), the average activity was again increased during the first hour as compared to the second one ($F(1,39)=201.6$, $p<0.001$), but without any difference between groups ($F(3,39)<1.0$ for the Group and the Interaction effects). On days of pre-exposure to ethanol (Day4 – Day 7), ANOVA showed significant main effects for Group ($F(3,39)=6.7$, $p<0.001$), Hour ($F(1,39)=92.2$, $p<0.001$) and for the Group×Hour interaction ($F(3,39)=25.6$, $p<0.001$). The Group effect was due to overall activity scores that were lower in rats given ethanol than in those given NaCl ($p<0.01$). The Group×Hour interaction was due to ethanol preventing the increase found after NaCl administration during the first hour, while activity levels during the second hour were not different among the four groups. It may be noteworthy that the activity levels recorded during the 2 h that preceded the injections were low and comparable among groups and days for the entire experimental period (in rats previously treated with NaCl, average hourly scores±S.E.M. were 5.49±0.7 for those given MDMA and 5.21±0.3 for those given EtOH+MDMA on the challenge day; in rats previously treated with EtOH, average hourly scores were 4.71±0.5 for those given MDMA and 4.94±0.4 for those given EtOH+MDMA on the challenge day).

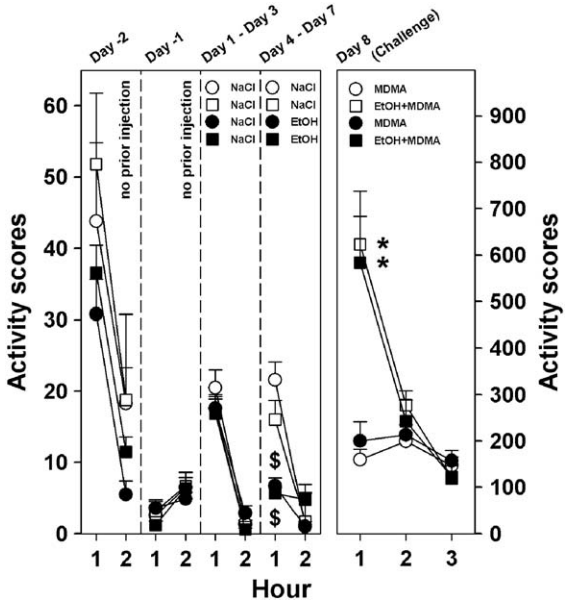

Fig. 2. Left panel: Locomotor activity scores recorded in the home cage during 2 consecutive hours (or first three post-injection hours on the challenge day) on the different testing days; Day −2: right after the rats were placed in the non familiar testing room on the activity recording devices; Day −1: same 2 h on the following day without any prior manipulation of the rats; Day 1 – Day 3: same 2 h after NaCl injections (values averaged over the 3 consecutive days of daily injections); Day 4 – Day 7: same 2 h after NaCl or EtOH injections (values averaged over the 4 consecutive days of treatment administration); Right panel: first 3 h after MDMA or EtOH+MDMA challenge (Day 8). Statistics: $significant activity reduction due to ethanol injections ($p<0.01$); *significant increase of activity as compared to MDMA alone ($p<0.001$). Note that the *Y*-scale on the challenge day (on the right) is much larger than that used for all previous days, due to MDMA effects. All data are means+S.E.M. Average hourly activity scores during the 2 h preceding the injections (i.e. about 5 cage crossings) were not different among groups and days (see Results).

To summarize, after familiarisation with the test conditions, rats reacted to NaCl injections by an increase of activity, and to ethanol injections by a decrease of activity, but these changes were observed only during the first post-injection hour.

On the challenge day (Day 8), all rats were given MDMA, in combination or not with ethanol. ANOVA of the corresponding activity scores showed no significant effect of ethanol Pre-exposure ($F(1,39)<1.0$), but there was a highly significant potentiating effect of ethanol on the locomotor response to MDMA ($F(1,39)=23.5$, $p<0.001$). There was also an overall Hour effect ($F(2,78)=31.1$, $p<0.001$), which was due to a progressive reduction of the overall activity scores (h_1 vs. h_2, $p<0.001$; h_3 vs. h_2, $p<0.01$), as well as a significant Ethanol co-administration × Hour interaction ($F(2,78)=27.3$, $p<0.001$), which was due to the fact that the Ethanol co-administration potentiated the effects of MDMA, but only during the first observation hour. All other interactions were not significant (Ethanol pre-exposure × Ethanol co-administration: $F(1,39)<1.0$; Hour × Ethanol pre-exposure: $F(2,78)<1.0$; Hour × Ethanol pre-exposure × Ethanol co-administration: $F(2,78)<1.0$).

3.2. Effects of EtOH and MDMA on temperature after prior ethanol experience

The data are shown in Fig. 3. Analysis of the temperatures recorded 1 h before drug injections did not show any significant inter-group difference, whether after the first or after the fourth injection. 1 h before the first injection, the average temperature was 37.3±0.1 °C. 1 h before the fourth injection, the temperature was of 37.4±0.1 °C (data not illustrated).

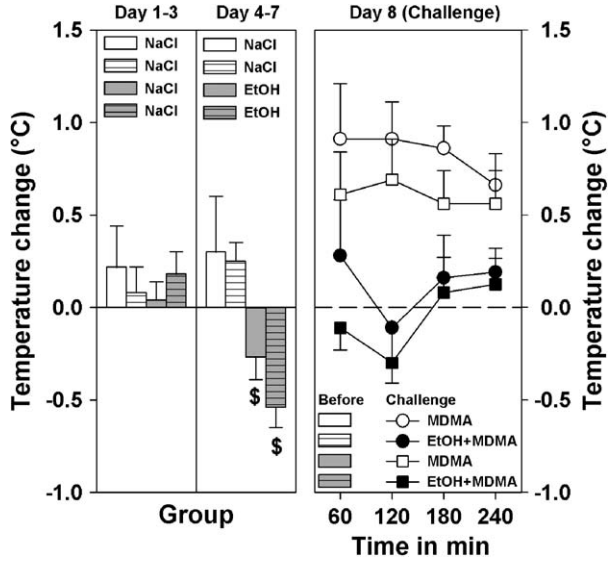

Fig. 3. Left panel: Body temperature changes recorded 30 min (Day 1 – Day 7) or over 4 h (challenge day) after injections of NaCl (Day 1 – Day 3), of NaCl or EtOH (Day 4 – Day 7), or of MDMA or EtOH+MDMA (Day 8). All data are means+S.E.M. Statistics: $ significant temperature reduction due to ethanol injections ($p<0.05$). Note that comparisons of temperature changes on the challenge day showed that rats given ethanol in addition to MDMA did not exhibit the overall temperature increase found in MDMA only rats, whether previously pre-exposed (average value on 4 h: +0.13±0.27 °C instead of +0.84±0.17 °C) or not (average: −0.05±0.08 °C instead of 0.61±0.14 °C) to ethanol ($p<0.01$ in each case). Absolute temperatures before the injections were about 37.4 °C (see Results) and did not differ among groups and days.

Analysis of the average temperature changes recorded during the first 3 days when NaCl was injected (Day 1 – Day 3) showed no significant Group effect ($F(3,28)<1.0$). Conversely, when half the rats were treated with ethanol during the next 4 days (Day 4 – Day 7), there was a significant Group effect ($F(3,28)=13.4$, $p<0.001$). Multiple comparisons showed that the temperatures in both groups of rats given ethanol were significantly lower than in those given NaCl injections ($p<0.001$). The temperature decrease after the first ethanol injection was −1.1±0.14 °C (vs. controls given NaCl) vs. −0.5±0.11 °C on the last day ($t=3.73$, $p<0.01$; data not illustrated). On the challenge day (Day 8), all rats were given MDMA, in combination or not with ethanol. ANOVA of the corresponding temperature changes showed no significant effect of Ethanol pre-exposure ($F(1,28)=1.3$), but there was a highly significant effect of Ethanol co-administration ($F(1,28)=14.0$, $p<0.001$), with rats having received ethanol in addition to MDMA showing temperatures that were significantly lower than in rats given MDMA only ($p<0.01$). Temperatures averaged over 4 h regardless of whether there was an ethanol pre-exposure were +0.72±0.1 °C in rats given only MDMA and +0.14±0.1 °C in those given ethanol in addition to MDMA. The Ethanol pre-exposure × Ethanol co-administration interaction was not significant ($F(1,28)<1.0$). Also the overall Hour effect ($F(3,84)<1.0$), the Ethanol co-administration × Hour interaction ($F(2,78)=27.3$, $p<0.001$), as well as all other interactions failed to reach significance (Hour × Ethanol pre-exposure: $F(3,84)<1.0$; Hour × Ethanol co-administration: $F(3,84)=2.3$, $p=0.08$; Hour × Ethanol pre-exposure × Ethanol co-administration: $F(3,84)<1.0$).

4. Discussion

MDMA induces locomotor hyperactivity and hyperthermia in rodents (e.g., Green et al., 2003). Although tolerance to MDMA effects has been reported (Parrott, 2005; but this review is mainly based on self reports in humans), we found that the effects on spontaneous activity and thermoregulation were relatively constant in magnitude when the same dose of MDMA was administered on 4 consecutive days (Cassel et al., 2004). Also, we recently reported, and herein confirm, that ethanol co-administered with MDMA potentiates the locomotor effects of MDMA, while, at the same time, attenuating its hyperpyretic effects (Cassel et al., 2004). As ethanol potentiated the locomotor – and thus psychostimulant – effects of MDMA, as it simultaneously attenuated the pyretic effects of MDMA, and as the potentiating effects of EtOH vanished within 2 h in rats given 10 mg/kg MDMA, whereas the preventive effects on temperature were still present 4 h after administration of an even lower dose of MDMA, we suggest that the mechanisms involved in these effects of ethanol are complex and can probably not be explained exclusively by pharmacokinetic factors. In addition, when measured 45 min after drug injection, we recently found that the MDMA and MDA content in the cortex, hippocampus and striatum were comparable in rats given 10 mg/kg MDMA or the same dose of MDMA+0.5 g/kg EtOH (Ben Hamida et al., unpublished data).

This observation, however, does not exclude the possibility of an ethanol-induced alteration of MDMA and/or metabolites brain penetration at earlier post-injection delays. Whatever may be, such data, which further support our previous observations, may have implications for health risks among polydrug abusers who combine ethanol and MDMA (but see Easton and Marsden, 2006, for caution about inter-species comparisons of MDMA effects). This may be particularly relevant because in humans, ethanol and MDMA co-administration is common, as indicated in various reports on polydrug usage including ecstasy (Lora-Tamayo et al., 2004; Pedersen and Skrondal, 1999; Schifano, 2004; Scholey et al., 2004). Based on our previous and current findings, ethanol may thus increase the acute psychostimulant effects of MDMA. Alternatively, the potential for ethanol to reduce the neurotoxic effects of MDMA cannot be ignored, although it has to be demonstrated yet. MDMA is toxic for serotonergic terminals (Green et al., 2003), and this toxicity is linked to several co-factors such as high ambient temperature, hyperthermia, free radicals formation (Farfel and Seiden, 1995; Johnson et al., 1995; Malberg et al., 1996; Malberg and Seiden, 1998; Sanchez et al., 2004). For instance, when the hyperpyretic effects of MDMA are impeded by low ambient temperature, MDMA does not induce serotonergic toxicity (e.g., Malberg and Seiden, 1998). In addition, preventive effects were reported for pentobarbitone (Colado et al., 1999) or dizocilpine (Farfel and Seiden, 1995) towards the MDMA-induced serotonergic toxicity, and this prevention was shown to be attributable to the pentobarbitone- or dizocilpine-induced hypothermia. Thus, as ethanol reduced the MDMA-induced hyperthermia in our rats, it seems possible that it will also attenuate the serotonergic toxicity of this drug. If so, combining MDMA with ethanol might be a way both to achieve psychostimulant effects at doses lower than in case of MDMA alone and, concomitantly, to attenuate the risk of serotonergic neurotoxicity. Although this issue deserves further investigation, two remarks may be worth mentioning. First, in a recent experiment, we found that repeated administrations of 6.6 mg/kg MDMA, in combination or not with ethanol, did not result in a significant reduction of serotonin concentrations in structures like the cortex or the hippocampus (unpublished data). Briefly, in this experiment, rats were injected with the drugs at four occasions. The delays between the injections were of 48 h, 120 h and 48 h, respectively. The rats were sacrificed 2 weeks after the last intoxication. Neither MDMA alone, nor MDMA + EtOH significantly affected the 5-HT concentration in the brain. It is noteworthy, however, that this experiment was performed in rats that were not exposed to ethanol before their first MDMA intoxication, and that the dose of MDMA may have been too weak, or the administrations too far apart, to induce serotonergic toxicity. Second, in a previous study in which rats were intoxicated daily with 10 mg/kg MDMA alone or in combination with 1.5 g/kg ethanol, but in which ethanol reduced hyperthermia only on the first day, the concentration of serotonin was reduced by about 30–35%, and this reduction was comparable whether rats had received ethanol or not in addition to MDMA (Cassel et al., 2005). Thus, the potential for ethanol to prevent the serotonergic toxicity of MDMA needs to be investigated using an appropriate injection schedule and high enough MDMA doses.

The pharmacokinetic and/or pharmacodynamic mechanisms underlying the ethanol-induced effects in MDMA-treated rats remain as yet unknown. Recently Johnson et al. (2004) showed that in mice given MDMA and ethanol, the striatal concentration of MDMA was 4 times greater than in those given only MDMA. This observation suggests that ethanol may also interfere with the metabolism of MDMA, or with its penetration in and/or elimination from the brain. Such a report would be more in favour of a pharmacokinetic interaction between ethanol and MDMA. Interestingly, along this line, previous studies suggested that ethanol and cocaine may interfere in a similar way, as the bioavailability of cocaine is increased by ethanol, and as brain concentrations of cocaine can be kept larger by ethanol, but only with a low dose and after a post-administration delay of more than 2 h (e.g., Pan and Hedaya, 1999). Ethanol may also directly interact with monoamine transporters, as it was shown to enhance the activity of the serotonin transporter in rat synaptosomes (Alexi and Azmitia, 1991) and of the human dopamine transporter expressed by *Xenopus* oocytes (Maiya et al., 2002; Mayfield et al., 2001), although at rather high concentrations. Further experiments are obviously needed to discover the pharmacology of the ethanol-induced modifications of MDMA effects.

In our previous experiment (Cassel et al., 2004), we found that the potentiation by ethanol of the stimulant effects of MDMA seemed to disappear when the treatment was repeated over four consecutive days, and also that the protective effects of ethanol on MDMA-induced hyperthermia were observed only on the first of these days. The latter observation was discussed initially in terms of rapid development of tolerance to ethanol (Cassel et al., 2004), and it was the aim of our present study to check whether the effects of ethanol on the physiological and behavioural responses to MDMA were still observed in rats that were not naïve to ethanol. Tolerance to ethanol, and particularly tolerance of the pyretic effects of ethanol (in general hypothermia at a normal ambient temperature; but see e.g., Myers, 1981), is described as a relatively fast phenomenon. For instance, such tolerance is observed when a second exposure to ethanol takes place within 24 to 48 h after the first one (Chan and York, 1994; Khanna et al., 1993, 1996). Our present results demonstrate that the rapid ethanol tolerance hypothesis, which we proposed to account for our former findings on the pyretic consequences of repeated EtOH + MDMA treatment, was in fact incorrect. Had it been true, pre-exposure to ethanol should have abolished the preventive effect of this drug on the MDMA-induced hyperthermia. Instead, repeated exposure to 1.5 g/kg ethanol alone, which resulted in the classically described body temperature decrease (and its attenuation with renewed administrations), did not alter the subsequent effects of ethanol combined with MDMA, whether on body temperature or on locomotor activity.

Also, prior treatment with ethanol did not alter the effects of MDMA when it was given alone on the challenge day. This observation may have some implications as regards cross-tolerance between drugs, especially because ethanol alone was shown to alter functional properties of the dopaminergic system, which is one of the target systems of MDMA. For instance, chronic ethanol intake may increase the number of D_1 receptor sites in the nucleus accumbens and thereby enhance the

behavioural response to a subsequent D-amphetamine challenge (Lograno et al., 1993). Changes in dopaminergic functions have also been described following short periods of exposure to ethanol (24 h to ethanol vapours), which revealed sufficient to induce both tolerance to ethanol-induced hypothermia and attenuation of amphetamine-induced release of dopamine by striatal synaptosomes (Mullin and Ferko, 1983). The status of the dopaminergic receptors or terminals was not assessed in the present study. Given that the response to MDMA was similar on the challenge day in rats pre-treated with ethanol and in their controls given saline pre-treatment instead, it seems reasonable to consider that our ethanol administration protocol did not affect dopaminergic functions (and this might be true also for serotonergic ones, another target for MDMA), at least not in a way that would result in altered responses to MDMA.

A last remark concerns the fact that our former experiment showed that EtOH only attenuated the hyperthermia due to MDMA, whereas prevention was complete in the current study. This difference in the magnitude of ethanol effects must be attributed to a difference in the magnitude of MDMA effects. Indeed, because we used a lower dose of MDMA (6.6 mg/kg, i.p. vs. 10 mg/kg, i.p.) in the present experiment, we produced less hyperthermia (about +0.7 °C vs. about +1.9 °C, 60 min after drug injection), which may have been easier to reverse by the same dose of EtOH. Another possible factor to account for this discrepancy may be related to differences in the ambient temperature, which is also known to exert dramatic influence on the magnitude of MDMA-induced hyperthermia (Green et al., 2004). Ambient temperature in the current study, however, was close to that used in our former study (the difference was of about 1 °C).

In conclusion, one has to keep in mind that our findings were obtained in rats. Although results obtained in animal models cannot be translated as such to humans (e.g., De la Torre and Farre, 2004; Easton and Marsden, 2006), they can generate valuable information on potential mechanisms, substrates or risks of drug interactions in humans. Interestingly, ethanol has been described to potentiate the subjective effects of MDMA in humans, subjects having taken ethanol and ecstasy reporting longer-lasting euphoria than those given MDMA alone (Hernandez-Lopez et al., 2002). This observation is parallel to our present findings on locomotor activity in rats, if one considers hyperactivity to account for psychostimulant effects. In so far as our results suggest, ethanol may both potentiate the psychostimulant effects of MDMA and prevent its hyperpyretic consequences. In addition, prior experience to what can be considered a moderately high dose of ethanol, at least when administered on four consecutive days, alters neither the potentiation of psychostimulant, nor the prevention of hyperthermic MDMA effects. Further studies should address whether this remains true after much longer periods of ethanol pre-exposure before MDMA and ethanol are taken in combination.

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