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ORIGINAL INVESTIGATION

MDMA attenuates THC withdrawal syndrome in mice

Clara Touriño · Rafael Maldonado · Olga Valverde

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Abstract
Introduction 3, 4-Methylenedioxymethamphetamine (MDMA) and cannabis are widely abused illicit drugs that are frequently consumed in combination. Interactions between these two drugs have been reported in several pharmacological responses observed in animals, such as body temperature, anxiety, cognition, and reward. However, the interaction between MDMA and cannabis in addictive processes such as physical dependence has not been elucidated yet.
Discussion In this study, the effects of acute and chronic MDMA were evaluated on the behavioral manifestations of Δ^9-tetrahydrocannabinol (THC) abstinence in mice. THC withdrawal syndrome was precipitated by injecting the cannabinoid antagonist rimonabant (10 mg/kg, i.p.) in mice chronically treated with THC and receiving MDMA (2.5, 5 and 10 mg/kg i.p.) or saline just before the withdrawal induction or chronically after the THC administration.
Results Both chronic and acute MDMA decreased in a dose-dependent manner the severity of THC withdrawal. In vivo microdialysis experiments showed that acute MDMA (5 mg/kg, i.p.) administration increased extracellular serotonin levels in the prefrontal cortex, but not dopamine levels in the nucleus accumbens. Our results also indicate that the attenuation of THC abstinence symptoms was not due to a direct interaction between rimonabant and MDMA nor to the result of the locomotor stimulating effects of MDMA.
Conclusion The modulation of the cannabinoid withdrawal syndrome by acute or chronic MDMA suggests a possible mechanism to explain the associated consumption of these two drugs in humans.

Keywords Psychostimulant · Cannabinoid · Physical dependence · In vivo microdialysis

Abbreviations

THC	Δ^9-tetrahydrocannabinol
DA	dopamine
5-HT	serotonin
NE	norepinephrine
MDMA	3, 4-methylenedioxymethamphetamine

Introduction

3, 4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a ring-substituted amphetamine derivative structurally related to hallucinogenic compounds. This widely abused illicit drug is mainly used among young people for recreational purposes (Green et al. 2003). Acute MDMA administration produces a rapid enhancement of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) release from nerve endings in several brain structures (Climko et al. 1986). In animal models, MDMA produces rewarding effects in different behavioral paradigms, such as the conditioned place preference (Bilsky et al. 1990) and the

C. Touriño · O. Valverde (✉)
Grup de Recerca de Neurobiologia del Comportament,
Departament de Ciències Experimentals i de la Salut,
Universitat Pompeu Fabra, PRBB,
C/ Dr. Aiguader 80,
08003 Barcelona, Spain
e-mail: olga.valverde@upf.edu

R. Maldonado
Laboratori de Neurofarmacologia,
Departament de Ciències de Experimentals i de la Salut,
Universitat Pompeu Fabra, PRBB,
C/ Dr Aiguader 80,
08003 Barcelona, Spain

intravenous self-administration paradigm (Beardsley et al. 1986; Trigo et al. 2006). However, no physical withdrawal syndrome has been found after a chronic treatment with MDMA (Robledo et al. 2004a). In humans, MDMA consumption is frequently associated with other drugs of abuse, mainly cannabis and alcohol (Smart and Ogborne 2000; Gouzoulis-Mayfrank and Daumann 2006).

Cannabis derivatives, whose main psychoactive constituent is Δ^9-tetrahydrocannabinol (THC; Mechoulam et al. 1970), are today the most consumed illicit drugs worldwide (Smart and Ogborne 2000). Two cannabinoid receptors, CB_1 (Matsuda et al. 1990) and CB_2 (Munro et al. 1993), have been identified. The CB_1 receptor is highly expressed in the central nervous system, and its stimulation mediates the psychotropic effects of cannabinoids (Ameri 1999; Maldonado and Rodríguez de Fonseca 2002). THC and other cannabinoid agonists have been reported to induce rewarding effects (Lepore et al. 1995; Valjent and Maldonado 2000; Justinova et al. 2005) and physical dependence in different animal models (Maldonado and Rodríguez de Fonseca 2002). Cannabinoid withdrawal is usually challenged in cannabinoid-dependent rodents by the administration of a CB_1 receptor antagonist and is characterized by the expression of a variety of somatic signs (Aceto et al. 1996; Hutcheson et al. 1998). In these conditions, animals exhibit several behavioral and motor somatic signs without relevant vegetative manifestations (Maldonado 2002; Lichtman and Martin 2002). Biochemical changes occurring during cannabinoid abstinence have also been related to the withdrawal syndrome of other drugs of abuse. These common changes include an increase in corticotrophin-releasing factor in the amigdaloid nuclei (Rodriguez de Fonseca et al. 1997), an up-regulation of adenylate cyclase/cyclic adenosine monophosphate signaling system in several brain areas (Hutcheson et al. 1998; Rubino et al. 2000), and decreased extracellular DA levels in limbic areas including the nucleus accumbens (Tanda et al. 1999).

There are several possible reasons for the frequent association between cannabis and ecstasy consumption in humans, such as the attenuation of the negative side effects associated to ecstasy consumption (Winstock et al. 2001; Zimmermann et al. 2005). Although individual effects of cannabis and MDMA have been widely studied, the effects of their combination are poorly understood. Despite the different mechanisms of action of these two drugs, both modulate common physiological processes such as locomotor activity, body temperature, stress-related responses and reward. Furthermore, MDMA and cannabis play an opposite role in the control of some of these physiological responses. Accordingly, THC administration prevents hyperthermia, hyperlocomotion, and anxiety-like responses produced by MDMA in rodents (Morley et al. 2004). However, the consequences of MDMA and THC association on addictive related responses have not been investigated yet. The present study evaluates the effects of acute and chronic administration of MDMA on the cannabinoid withdrawal syndrome in mice. The observed behavioral and neurochemical changes demonstrate for the first time that MDMA attenuates the severity of THC abstinence.

Materials and methods

Animals

Male CD1 mice (Charles River, France) weighing 22–24 g at the beginning of the study were housed five per cage in a temperature (21±1°C) and humidity-controlled (55±10%) room with a 12/12-h light-dark cycle (light on between 0800 and 2000 hours). Food and water were available ad libitum. The mice were habituated to their new environment and kindly handled for 1 week before starting the experiments. Animal procedures were conducted in accordance with the guidelines of the European Communities Directive 86/609/EEC regulating animal research and approved by the local ethical committee (CEEA IMAS-UPF). Investigators were blind to the treatment conditions.

Drugs

MDMA hydrochloride was obtained from Lipomed, A.G. (Arlesheim, Switzerland) and dissolved in 0.9% physiological saline. THC (THC Pharm, Frankfurt, Germany) was dissolved in a solution of 5% ethanol, 5% cremophor EL (Sigma Chemical, Madrid, Spain) and 90% distilled water. MDMA and THC were administered by intraperitoneal route (i.p.) in a volume of 0.1 ml/10 g. The selective CB_1 receptor antagonist rimonabant (kindly gifted by Sanofi-Aventis, Montpellier, France) was dissolved in a solution of 10% ethanol, 10% cremophor EL and 80% distilled water, and administered by i.p. route in a volume of 0.2 ml/10 g.

THC dependence and withdrawal

THC dependence was induced as previously reported (Valjent and Maldonado 2000). Briefly, the mice were injected with THC (20 mg/kg, i.p.) or vehicle twice daily at 10:00 and 20:00 hours during five consecutive days. On the 6th day, the mice received only the THC morning injection. Then, to precipitate the THC withdrawal syndrome, the animals received rimonabant (10 mg/kg, i.p.) 4 h after the last THC injection. In addition, 60 min before receiving rimonabant, the animals also received a single dose of MDMA (2.5, 5, and 10 mg/kg, i.p.) or saline to evaluate the

effects of acute MDMA on THC abstinence. The different doses of MDMA were evaluated in separate experiments. To evaluate the effects of the chronic administration of MDMA on THC withdrawal, the animals received an injection of MDMA (2.5, 5, and 10 mg/kg, i.p.) or saline 1 h after every THC administration. The different doses of MDMA (2.5, 5, and 10 mg/kg) were evaluated in the same experiment.

Evaluation of the somatic expression of THC withdrawal

THC withdrawal was evaluated as previously reported (Hutcheson et al. 1998). Mice were placed in a circular clear plastic observation area (30 cm diameter×35 cm height) for a 5-min period of habituation. Immediately after habituation, the animals received an injection of rimonabant (10 mg/kg, i.p.) or appropriate vehicle. The mice were then observed for a 45-min period. Observations of somatic signs after rimonabant challenge were divided in 5-min time intervals. The number of bouts of writhing, wet dog shake, sniffings, and front paw tremor were counted. Penile licking or erection, ataxia, hunched posture, tremor, ptosis, diarrhea, mastication, and piloerection were scored for appearance or nonappearance within each 5-min time epoch. Scores for the level of activity were made by giving in each 5-min period a value of 0=low activity, 1=normal activity, or 2=increased activity. A global withdrawal score was calculated for each animal by giving each individual sign a relative weight, as reported previously. Global withdrawal score values range from 0 to 100 (Valverde et al. 2000).

Body temperature

Rectal temperature was measured in each mouse by placing 3 cm of an electronic thermocouple flexible rectal probe (Panlab, Madrid, Spain) in the mice rectum for 10 s. Basal temperature was measured just before the last THC injection. Temperature was also evaluated 30 min after the injection of rimonabant. Data are represented as the difference in rectal temperature 30 min after rimonabant injection and basal temperature.

Locomotor activity

The locomotor response induced by an acute MDMA injection was evaluated by using individual locomotor activity boxes (9×20×11 cm; Imetronic, Lyon France) in a low luminosity room (5 lx), and with white noise. The boxes were provided with two lines of 14 photocells each, one 2 cm above the floor to measure horizontal activity and other located 6 cm above the floor to measure vertical activity (rears). Locomotor activity was measured as the number of beam breaks during 1-h period. One hour after MDMA (5 or 10 mg/kg, i.p.) or saline administration, the animals received acute rimonabant (10 mg/kg, i.p.) or vehicle and were immediately placed in the locomotor activity boxes during 1 h.

Microdialysis procedure

Microdialysis studies were performed as previously described (Robledo et al. 2004b). Mice were injected with THC (20 mg/kg, i.p.) twice daily at 10:00 and 20:00 hours for 5 days to induce THC dependence. On day 4, the animals received the THC morning injection and 2–6 h later were anesthetized with a ketamine/xylazine mixture and placed in a stereotaxic apparatus with a flat skull (Paxinos and Franklin 1997). A small hole was drilled on the right side of the skull, and the analytical probe was inserted in the prefrontal cortex (anteroposterior, +2.2 mm; mediolateral, −0.5 mm; dorsoventral, −3.0 mm from bregma; CMA/7 7/2 mm; CMA Microdialysis) or the nucleus accumbens (anteroposterior, +1.6 mm; mediolateral, −0.9 mm; dorsoventral, −3.6 mm from bregma; CMA/7 7/1 mm; CMA Microdialysis) and then fixed to the skull with dental cement (Dentalon Plus, Heraeus Kulzer, Germany). One day after probe implantation, the animals were habituated to the experimental environment overnight. The following morning, Ringer's solution was pumped through the dialysis probe at a constant rate of 1 μl/min. The animals received the morning injection of THC, and 1 h and 40 min later, five consecutive 20-min dialysis samples were collected for the determination of baseline 5-HT or DA levels. Then, mice were injected with saline or MDMA 5 mg/kg 3 h after THC injection, and three consecutive 20-min dialysis samples were collected for the determination of MDMA effects on THC-dependent animals. Four hours after THC administration, the animals were challenged with rimonabant (10 mg/kg), and samples were collected every 20 min for 4 h. Dialysate samples (15 μl) were injected without any purification into a high performance liquid chromatography (HPLC) system that consisted of a pump linked to an automatic injector (Agilent 1100; Agilent Technologies, Palo Alto, CA), a reverse-phase column (Zorbax SB C18, 3.5 μm, 150×4.6 mm; Agilent Technologies), and a coulometric detector (Coulochem II; CMA Microdialysis, North Chelmsford, MA) with a 5011A analytical cell. To quantify DA, the first electrode was fixed at −100 mV and the second electrode at +300 mV. The composition of the mobile phase was 50 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.65 mM octane sulfonic acid, and 15% (v/v) methanol, pH 3.5. To quantify 5-HT, the first electrode was fixed at +50 mV and the

second electrode at +300 mV. The composition of the mobile phase was 50 mM $CH_3CO_2Na{\cdot}3H_2O$, 0.1 mM Na_2EDTA, 0.65 mM octane sulfonic acid, and 25% (v/v) methanol, pH 5.0.

Histology

At the end of the experiments, the animals were killed, and the brains were quickly removed and stored at −80°C to check the position of the probe. The brains were cut using a cryostat in 20 μm serial coronal sections, which were then processed with cresyl violet and observed under a microscope. Only those animals that had been implanted correctly were included in the study.

Statistical analysis

Effects of acute MDMA on the somatic signs of withdrawal were compared by using a one-way analysis of variance (ANOVA) for treatment followed by post hoc comparisons (Scheffe's test). Effects of chronic MDMA on the somatic signs of withdrawal were analyzed by using a two-way ANOVA with treatment (THC or vehicle, and MDMA or saline) as between-subjects factors of variation, followed by corresponding one-way ANOVA and post hoc comparison (Dunnett's test). Extracellular 5-HT levels in the prefrontal cortex and DA levels in the nucleus accumbens were analyzed by using a two-way ANOVA with treatment (MDMA or saline) and time as between and within factors of variation, respectively. Unpaired two-tailed *t* test was calculated to compare the effect of MDMA vs saline treament. Locomotor activity was analyzed using two-way ANOVA with treatments (rimonabant or vehicle, and MDMA or saline) as between-subjects factors of variation, followed by corresponding one-way ANOVA and post hoc comparison (Dunnett's test). Body temperature values were compared by using a one-way ANOVA for treatment followed by post hoc comparisons (Dunett's test). ED_{50} value was determined by nonlinear regression analysis of the dose-response curves using PRISM (GraphPad, San Diego). Differences were considered significant if the probability of error was less than 5%.

Results

Acute MDMA attenuated THC withdrawal syndrome

The severity of rimonabant-precipitated THC withdrawal syndrome was evaluated in chronically THC or vehicle-treated mice. Withdrawal syndrome was challenged with rimonabant (10 mg/kg, i.p.) or its corresponding vehicle. These animals also received acute MDMA (2.5, 5 and 10 mg/kg) or saline 1 h before rimonabant challenge. The abstinence of some THC-dependent mice was triggered with vehicle. These animals exhibited global withdrawal score values similar to the chronically vehicle-treated mice (THC + MDMA 2.5 mg/kg=15.3±1.6, THC + MDMA 5 mg/kg=14.8±1.6, THC + MDMA 10 mg/kg=18.3±3.3). However, the severity of THC withdrawal syndrome precipitated by rimonabant in THC-dependent mice was attenuated in a dose-dependent manner by acute administration of MDMA. The estimated ED_{50} value (95% confidence limits) calculated for acute MDMA administration on THC abstinence was 4.96 mg/kg (4.81–5.11). This drug reduced not only the global withdrawal score, but also the severity of individual signs of abstinence (Table 1; Fig. 1).

Statistical analysis indicated that MDMA, at the dose of 2.5 mg/kg, did not produce changes in the manifestations of THC withdrawal signs (Table 1). One-way ANOVA, calculated for the global withdrawal score, revealed a significant treatment effect on the abstinence severity ($F_{(3,\ 36)}=8.88$, $p<0.001$). Subsequent post hoc analysis showed a strong withdrawal syndrome in the THC-abstinent group ($p<0.01$) that was not significantly modified by the administration of MDMA at the dose of 2.5 mg/kg (Fig. 1a).

MDMA (5 mg/kg) significantly attenuated the severity of THC withdrawal as observed when the individual somatic signs of abstinence were analyzed. Post hoc analysis calculated for the different abstinence signs revealed that the administration of MDMA (5 mg/kg) to THC-dependent mice produced a significant decrease in several signs, such as tremor ($p<0.01$), ptosis ($p<0.05$), and mastication ($p<0.01$) (Table 1). Moreover, one-way ANOVA calculated for the global withdrawal score values indicated a significant treatment effect ($F_{(3,\ 29)}=22.87$, $p<0.001$). Subsequent post hoc analysis showed that MDMA (5 mg/kg) significantly reduced the severity of withdrawal on THC-dependent mice ($p<0.01$; Fig. 1b).

MDMA administered at the dose of 10 mg/kg suppressed the manifestations of THC withdrawal syndrome. Indeed, MDMA (10 mg/kg) produced a significant reduction in 8 over the 13 abstinence signs evaluated: wet dog shakes ($p<0.001$), sniffing ($p<0.05$), writhing ($p<0.05$), tremor ($p<0.05$), ptosis ($p<0.001$), mastication ($p<0.001$), hunched posture ($p<0.05$), and penile licking ($p<0.05$). A trend to attenuate other withdrawal signs (paw tremor, diarrhea, and ataxia) was also observed. This attenuation contributed for the decreased global withdrawal score values produced by MDMA administration (Table 1). At the same time, one-way ANOVA calculated for the global withdrawal score revealed a significant treatment effect ($F_{(3,\ 58)}=36.55$, $p<0.001$). Therefore, the global withdrawal

Table 1 Effects of acute MDMA (2.5, 5, and 10 mg/kg i.p.) or saline, on THC-dependent mice, administered 1 h before abstinence

	Vehicle		THC		Vehicle		THC		Vehicle		THC	
	Saline	MDMA 2.5	Saline	MDMA 2.5	Saline	MDMA 5	Saline	MDMA 5	Saline	MDMA 10	Saline	MDMA 10
Locomotor activity	7.9±0.5	8.9±1.0	9.4±0.7	8.0±0.6	7.4±0.6	12.3±1.2	8.9±1.3	12.4±1.2	8.2±0.7	11.3±1.1	7.6±1.1	14.7±1.1***
Wet dog shakes	4.1±1.1	9.9±3.3	23.8±4.3	21.5±4.7	2.0±0.7	1.9±0.8	29.6±4.4	18.9±3.9	5.1±1.6	1.4±0.5	29.7±4.9	5.6±1.7***
Paw tremor	0.3±0.1	3.3±0.8	21±6.1	20.6±6.1	0.4±0.3	1.3±0.6	29.6±9.9	8.7±1.6	0.3±0.2	2.1±1.0	18.0±3.9	8.1±2.9
Sniffings	0.2±0.1	1.2±0.5	44.4±17.3	25.5±8.6	0.0±0.0	0.0±0.0	33.2±10.5	10.9±4.8	0.4±0.2	0.5±0.3	15.2±4.5	4.2±0.9*
Writhings	0.8±0.3	0.9±0.6	4.9±2.0	9.0±6.7	0.3±0.3	1.9±1.4	0.8±0.6	0.3±0.2	1.1±0.5	0.2±0.1	11.1±4.6	0.2±0.1*
Tremor	2.7±0.6	4.9±0.7	6.6±0.5	5.9±0.9	2.7±0.8	0.9±0.3	7.1±0.5	3.8±0.7**	2.6±0.8	1.4±0.1	6.8±0.3	4.3±0.7*
Ptosis	4.0±0.6	5.4±0.9	6.8±0.5	4.1±1.1	5.1±0.9	2.0±0.7	7.6±0.4	3.6±1.1*	3.4±0.8	2.0±0.5	6.5±0.7	1.3±0.6***
Diarrhea	0.4±0.2	0.8±0.4	1.8±0.6	1.9±0.6	0.0±0.0	0.1±0.1	1.2±0.4	0.3±0.2	0.6±0.3	0.3±0.2	1.4±0.3	0.6±0.3
Mastication	0.9±0.3	2.4±0.6	5.1±0.8	4.9±0.8	0.6±0.4	0.3±0.2	6.1±0.6	2.7±0.8**	1.0±0.5	1.3±0.7	5.8±0.5	2.4±0.6**
Piloerection	4.2±0.7	6.0±0.8	7.9±0.3	6.8±0.9	4.4±1.1	6.0±1.2	7.6±0.5	8.1±0.4	4.1±0.9	5.4±0.9	7.4±0.6	8.7±0.2
Ataxia	0.9±0.2	1.6±0.3	1.9±0.4	2.2±0.4	1.4±0.4	2.0±1.0	1.6±0.4	1.6±0.4	0.6±0.2	0.2±0.1	3.8±0.8	1.9±0.6
Hunched posture	2.9±0.5	5.0±0.8	4.4±0.8	5±0.8	5.0±0.8	1.3±0.4**	6.1±0.6	6.0±0.6	1.9±0.5	0.9±0.4	4.1±0.7	1.4±0.5*
Penile licking	2.0±0.3	2.7±0.6	2.1±0.6	2.3±0.8	2.4±0.5	1.7±0.6	1.3±0.5	2.8±0.8	1.8±0.4	1.1±0.4	2.8±0.7	0.8±0.2*

THC withdrawal syndrome was precipitated by rimonabant (10 mg/kg, i.p.) administration. Data are expressed as mean±SEM. Vehicle saline, n=7–21 mice; vehicle MDMA n=7–15; THC saline n=9–17; THC MDMA n=10–16 mice.

*p<0.05 when comparing THC + MDMA group with THC + Vehicle group

**p<0.01 when comparing THC + MDMA group with THC + Vehicle group

***p<0.001 when comparing THC + MDMA group with THC + Vehicle group

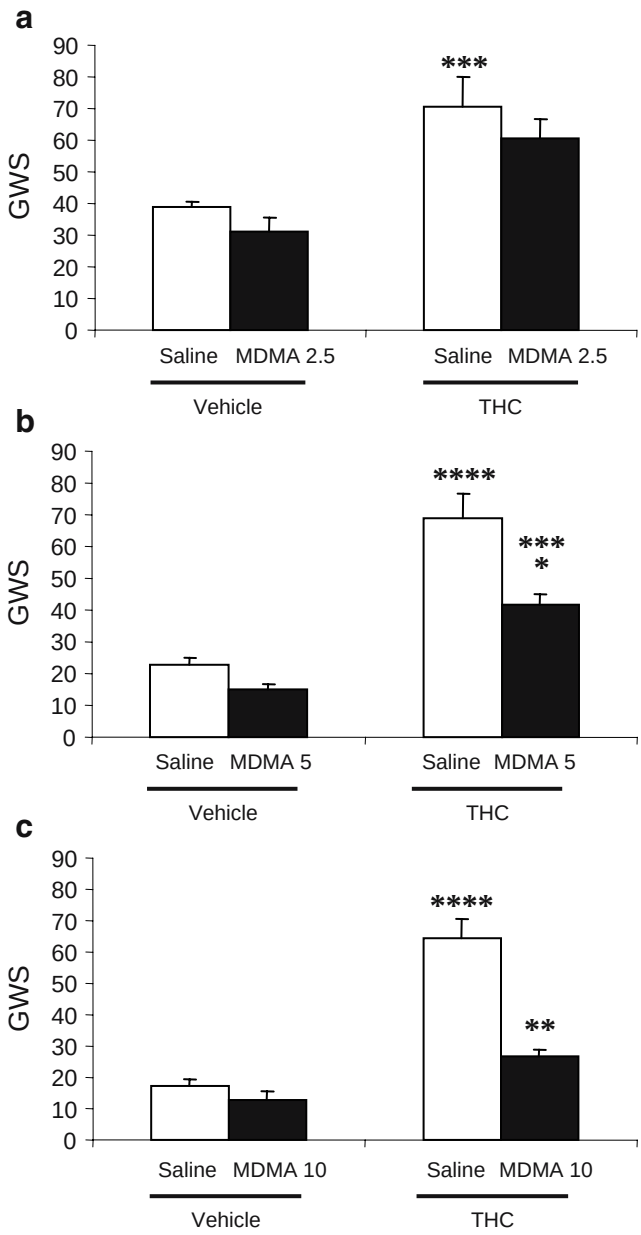

Fig. 1 Rimonabant-precipitated THC withdrawal syndrome in mice pretreated with acute MDMA (i.p.) 2.5 (**a**); 5 (**b**), and 10 (**c**) mg/kg, or saline. Abstinence was precipitated by acute rimonabant injection (10 mg/kg, i.p.) in THC-dependent mice (see Materials and methods). A global withdrawal score (*GWS*) was calculated for each animal by giving to each individual sign a relative weight (see Materials and methods). Data are expressed as mean±SEM (vehicle saline, n=7–21 mice; vehicle MDMA n=7–15; THC saline n=9–17; THC MDMA n=10–16 mice). *p<0.01; **p<0.001 when comparing between abstinent animals. ***p<0.01; ****p<0.001 when compared with vehicle–saline group (Scheffe test)

score was dramatically decreased after MDMA (10 mg/kg) administration in THC-abstinent mice (p<0.001), exhibiting similar score values than vehicle-treated groups (Fig. 1c).

Body temperature decrease was evaluated in THC-dependent (−2.1±0.2°C) and nondependent (−1.0±0.3°C) animals after being challenged with a rimonabant injection. Unpaired Student's t test analysis indicated a significant hypothermia in THC-dependent mice (p<0.01). The effect of acute MDMA (5 and 10 mg/kg i.p.) or saline was also evaluated in THC-abstinent mice (THC + MDMA 5 mg/kg=−1.8±0.2; THC + MDMA 10 mg/kg=−1.3±0.3). One-way ANOVA showed no effect of MDMA treatment ($F_{(2,\ 53)}$=1.71, n.s.) on the hypothermia observed in THC-abstinent mice. Thus, administration of MDMA did not modify hypothermia produced by THC withdrawal syndrome.

Chronic MDMA attenuated THC withdrawal syndrome

THC-dependent mice chronically treated with MDMA (2.5, 5 and 10 mg/kg twice a day) showed an attenuation in the severity of withdrawal syndrome triggered by rimonabant. Statistical analysis indicated that chronic administration of MDMA also reduced dose-dependently the manifestations of THC withdrawal (Fig. 2, Table 2). Two-way ANOVA, calculated for the global withdrawal score, revealed a significant effect of THC treatment ($F_{(1,\ 59)}$=86.16, p<0.001), MDMA treatment ($F_{(3,\ 59)}$=10.94, p<0.001), and interaction between both factors ($F_{(1,\ 59)}$=6.27, p<0.01). Subsequent one-way ANOVA indicated a significant effect of MDMA treatment on both vehicle ($F_{(3,\ 19)}$=3.50, p<0.05) and THC-treated mice ($F_{(3,\ 40)}$=18.43, p<0.001). Post hoc analysis showed a dose-dependent reduction in global withdrawal score in THC-treated animals when compared to the saline-treated group (MDMA 2.5 mg/kg, p<0.01; MDMA 5 mg/kg, p<0.001; MDMA 10 mg/kg, p<0.001), but this effect was not observed in vehicle-treated mice. One-way ANOVA (THC treatment) showed significant differences in the global

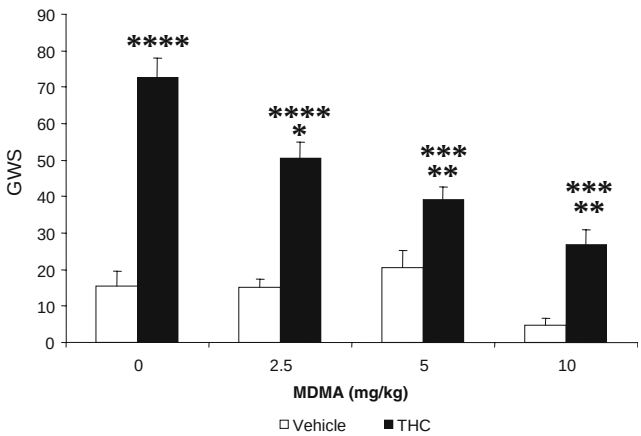

Fig. 2 Rimonabant-precipitated THC withdrawal syndrome in mice chronically pretreated with MDMA (2.5, 5, and 10 mg/kg, i.p.) or saline. Data are expressed as mean±SEM (vehicle groups, n=4–5 mice; THC groups n=10 mice). *p<0.01; **p<0.001, when compared with vehicle-treated group between abstinent animals. ***p<0.01; ****p<0.001 when compared with saline-treated group (Dunnett's test)

Table 2 Effects of chronic MDMA (2.5, 5, and 10 mg/kg, i.p.) or saline, on THC-dependent mice, administered 1 h after THC

	Vehicle				THC			
Individual signs	Saline	MDMA 2.5	MDMA 5	MDMA 10	Saline	MDMA 2.5	MDMA 5	MDMA 10
Locomotor activity	9.7±1.3	8.8±0.3	7.8±0.7	8.9±0.5	8.0±0.5	8.0±1.4	8.1±1.0	8.3±0.7
Wet dog shakes	15.4±8.2	4.3±1.1	12.8±3.7	4.6±1.4	35.2±4.8	22.4±5.0*	17.4±3.8$^{\#}$	18.3±4.3$^{\#}$
Paw tremor	0.6±0.4	0.0±0.0	3.6±1.2$^{\#}$	0.8±0.6	38.8±9.8*	29.1±11.0	17.9±6.4	12.7±5.5
Sniffings	0.0±0.0	0.3±0.3	1.4±1.4	0.0±0.0	16.8±7.6	6.1±2.4	5.4±2.5	1.3±0.8$^{\#}$
Writhings	6.6±4.2	1.0±1.0	3.6±1.8	1.2±1.0	12.2±3.2	8.0±4.1	12.4±7.3	0.3±0.2
Tremor	0.6±0.4	1.5±1.0	0.8±0.6	0.4±0.2	5.6±1.0**	3.4±0.8	2.2±0.6$^{\#}$	2.2±0.9$^{\#}$
Ptosis	2.8±1.5	2.8±1.1	4.0±1.0	0.0±0.0	7.5±0.6**	7.0±0.7**	5.5±0.9	3.0±1.0$^{\#\#\#}$
Diarrhea	0.0±0.0	1.3±0.9	0.4±0.4	0.0±0.0	0.8±0.5	0.1±0.1	0.8±0.5	0.2±0.2
Mastication	0.2±0.2	0.3±0.3	0.2±0.2	0.2±0.2	5.8±0.7***	4.2±0.8*	1.9±0.7$^{\#\#\#}$	0.9±0.3$^{\#\#\#}$
Piloerection	1.4±0.9	6.3±1.1$^{\#}$	3.0±1.8	0.0±0.0	7.3±0.4***	7.2±0.8	6.2±0.8	4.4±0.7$^{\#}$**
Ataxia	1.6±0.4	0.8±0.3	2.6±0.5	0.8±0.4	4.1±0.7*	2.4±0.4$^{\#\#}$	1.8±0.2$^{\#}$	1.9±0.3$^{\#}$*
Hunched posture	0.2±0.2	1.5±1.5	1.2±1.2	0.8±0.6	2.5±1.0	1.5±0.8	0.9±0.6	1.9±0.8
Penile licking	0.2±0.2	0.3±0.3	1.2±1.0	0.2±0.2	1.2±0.6	1.1±0.6	0.5±0.4	0.7±0.3

THC withdrawal syndrome was precipitated by rimonabant (10 mg/kg, i.p.) administration. Data are expressed as mean±SEM. Vehicle group, n=4–5 mice; THC group n=10 mice.

*p<0.05 when compared with the vehicle group of the same MDMA dose

**p<0.01 when compared with the vehicle group of the same MDMA dose

***p<0.001 when compared with the vehicle group of the same MDMA dose

$^{\#}p$<0.05 when compared with the saline group of the same vehicle or THC group (Dunnett's test)

$^{\#\#}p$<0.01 when compared with the saline group of the same vehicle or THC group (Dunnett's test)

$^{\#\#\#}p$<0.001 when compared with the saline group of the same vehicle or THC group (Dunnett's test)

withdrawal score between vehicle- and THC-treated mice, and indicates that chronic MDMA administration reduced in a dose-dependent way the severity of THC abstinence [saline ($F_{(1,\ 15)}$=45.53, p<0.001); MDMA 2.5 mg/kg ($F_{(1,\ 14)}$= 22.24, p<0.001); MDMA 5 mg/kg ($F_{(1,\ 15)}$=9.25, p<0.01); MDMA 10 mg/kg ($F_{(1,\ 15)}$=12.05, p<0.01)].

Increase in 5-HT release in the prefrontal cortex but not DA release in the nucleus accumbens after MDMA administration in THC-dependent mice

Extracellular levels of 5-HT in the prefrontal cortex were evaluated in THC-abstinent animals treated with MDMA 5 mg/kg or saline (Fig. 3). Two-way ANOVA showed a significant effect of MDMA treatment ($F_{(1,\ 12)}$=58.14, p<0.001), time ($F_{(19,\ 228)}$=25.67, p<0.001), and interaction between time and treatment ($F_{(19,\ 228)}$=29.37, p<0.001). Subsequent one-way ANOVA for time indicated that MDMA produced significant increase in 5-HT release in prefrontal cortex ($F_{(19,\ 133)}$=41.78, p<0.001), but no effect of rimonabant was observed in animals treated with saline ($F_{(19,\ 95)}$=1.20, n.s.). 5-HT release in the prefrontal cortex significantly increased for 220 min after MDMA injection when compared with saline group. Extracellular levels of DA in the nucleus accumbens were also evaluated (Fig. 3). Two-way ANOVA showed a significant effect of time ($F_{(19,\ 171)}$=2.54, p<0.001), interaction between time and treatment ($F_{(19,\ 171)}$=2.67, p<0.001), but not treatment effect ($F_{(1,\ 9)}$=4.75, n.s.). These results show that MDMA does not significantly increase DA in the nucleus accumbens. In addition, rimonabant administration did not modify DA extracellular levels in the nucleus accumbens in THC-dependent mice ($F_{(19,\ 76)}$=0.81, n.s.).

Rimonabant did not modify hyperlocomotion induced by MDMA

The possible interaction between acute MDMA (5 and 10 mg/kg i.p.) and rimonabant (10 mg/kg i.p.) on locomotor activity was evaluated. Two-way ANOVA indicated a main hyperlocomotor effect of MDMA treatment ($F_{(2,\ 74)}$=42.38, p<0.001), without rimonabant effect or interaction between these two factors. Thus, administration of rimonabant did not modify MDMA hyperlocomotor effects or had any intrinsic effect on locomotion (Fig. 4).

Discussion

This study demonstrates for the first time that MDMA attenuates the physical somatic signs of withdrawal in THC-dependent mice. MDMA tested at three different doses (2.5, 5 and 10 mg/kg), produced a dose-dependent decrease in THC withdrawal syndrome, when administered both acutely and chronically (Figs. 1 and 2). This effect does not seem to be related to an increase in locomotion,

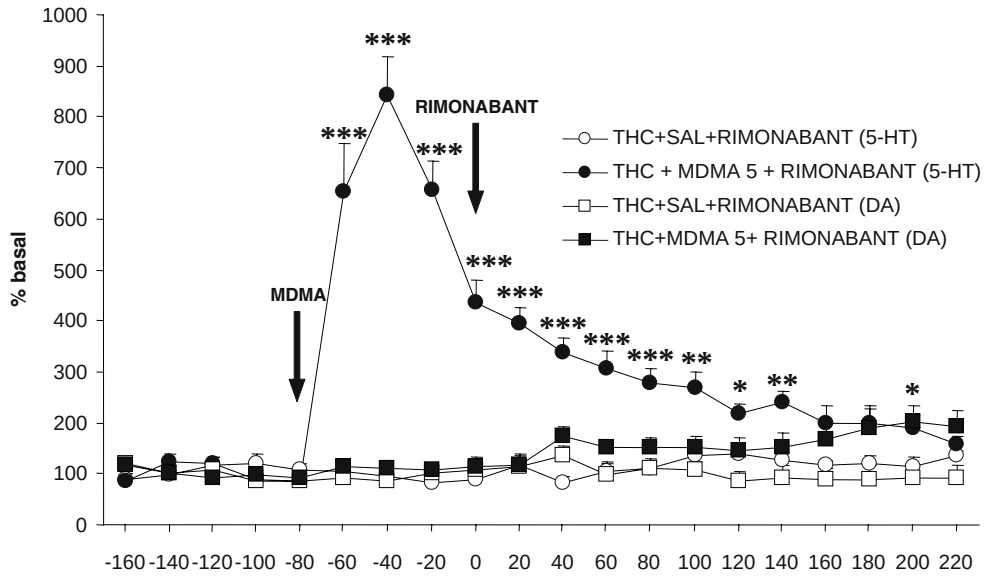

Fig. 3 Effects of MDMA (5 mg/kg, i.p.) or saline on 5-HT or DA concentrations in dialysates obtained by in vivo microdialysis from the prefrontal cortex or the nucleus accumbens, respectively, in THC-dependent mice. The *arrows* indicate MDMA or saline injection at time −80 min and rimonabant (10 mg/kg i.p.) injection at time 0. Dialysate samples were collected every 20 min during 2 h 40 min before and 3 h 40 min after rimonabant injection. 5-HT absolute levels were 1.4±0.2 pg/15 µl for the saline-treated group and 1.1±0.1 pg/15 µl for the MDMA-treated group. DA absolute levels were 3.4±0.6 pg/15 µl for the saline-treated group and 2.6±0.4 pg/15 µl for the MDMA-treated group. All values are expressed as mean±SEM (n=5–6 for DA and n=6–8 for 5-HT experiments). *p<0.05, **p<0.01; ***p<0.001 when compared with saline-treated group (unpaired two-tailed t test)

since MDMA at the doses that attenuated cannabinoid withdrawal syndrome did not induce significant hyperlocomotion. In addition, nonmotor-related signs of withdrawal, such as diarrhea, ptosis, tremor, or wet dog shakes were also significantly reduced by MDMA administration. The attenuation of cannabinoid withdrawal by a direct interaction between the CB_1 receptor antagonist rimonabant and MDMA can be discarded, since no modification on MDMA-induced hyperlocomotion was observed when these two drugs were simultaneously administered (Fig. 4). In agreement, rimonabant did not modify changes in locomotion induced by other psychostimulants, such as cocaine (Lesscher et al. 2005).

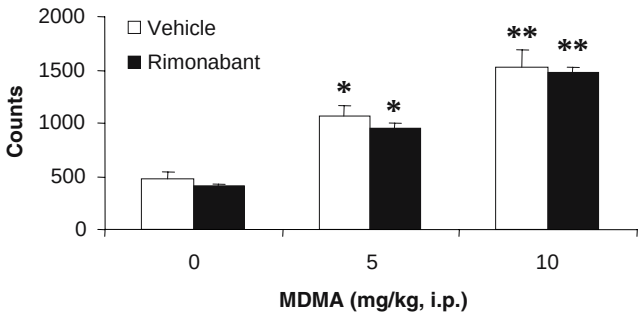

Fig. 4 Effects of acute rimonabant on hyperlocomotion induced by MDMA. Rimonabant (10 mg/kg, i.p.; *black bars*) or vehicle (*white bars*) were administered 1 h after MDMA (5 or 10 mg/kg, i.p.) or saline treatment. Data are expressed as mean±SEM of total counts during a 1-h period. n=7–9 mice per group. *p<0.01; **p<0.001 when compared with saline treated group (Dunnett's test)

Several studies have reported a pharmacological interaction between cannabinoids and MDMA. The cannabinoid agonists CP55940 and THC reduced MDMA-induced hyperlocomotion, hyperthermia, and anxiety-like behavior in rats (Morley et al. 2004). Moreover, coadministration of THC and MDMA has been reported to synergistically disrupt working memory in rats (Young et al. 2005). In addition, CP55940 administration reduced MDMA self-administration in rats (Braida and Sala 2002), suggesting that cannabinoids also modulate MDMA reinforcing properties.

MDMA could reduce the severity of THC withdrawal syndrome by attenuating somatic signs related to hypothermia and locomotor alterations. In our experimental conditions THC-abstinent mice exhibited a significant decrease in body temperature that could contribute to the appearance of some somatic signs related to hypothermia such as tremor, wet dog shakes, or piloerection. No significant effect of MDMA but a trend was observed reducing hypothermia produced during THC abstinence. Therefore, MDMA-induced hyperthermia does not seem to play a key role in the observed attenuation of THC withdrawal. However, a small contribution on the reduction in the somatic signs of THC abstinence cannot be discarded. Motor somatic signs of THC withdrawal have been attributed to adaptive changes occurring in the brain during

cannabinoid chronic administration (Hutcheson et al. 1998). On the one hand, the endocannabinoid system is involved in the motor control (Steiner et al. 1999) and CB_1 receptors are highly expressed in the brain motor areas including the cerebellum (Herkenham et al. 1990). Besides, MDMA notably increases the release of DA and 5-HT (Johnson et al. 1986) in the striatum (Yamamoto and Spanos 1988) and cortex (Colado et al. 1993). The data obtained when measuring extracellular 5-HT levels in the prefrontal cortex and DA levels in the nucleus accumbens showed that MDMA significantly increased 5-HT in prefrontal cortex but not DA in the nucleus accumbens in mice. Therefore, we propose that the elevation of 5-HT release in the prefrontal cortex produced by MDMA plays a crucial role in the observed attenuation of cannabinoid abstinence. Although DA levels in the nucleus accumbens were not increased, a possible role for this neurotransmitter cannot be fully discarded. Specifically, differences in DA turnover could contribute to the observed effects. Furthermore, rimonabant does not alter 5-HT or DA levels in animals treated with saline or MDMA, showing that the effect of MDMA on THC withdrawal syndrome is not due to a direct interaction with the CB_1 receptor antagonist. Hence, MDMA could attenuate THC withdrawal syndrome by modulating the functional activity of motor brain areas including cortex, basal ganglia, or cerebellum, whose function could have been altered by chronic exposure to THC. Thus, the increase in monoamine produced by MDMA could compensate an abnormal 5-HT transmission in motor brain areas on THC-abstinent mice. This alteration in the 5-HT transmission seems to take place during the development of cannabinoid dependence, since rimonabant administration did not produce changes in 5-HT levels in the prefrontal cortex of THC-dependent animals. Accordingly, MDMA has been reported to reduce dyskinesias in a marmoset model of Parkinson's disease, through a 5-HT-mediated mechanism (Iravani et al. 2003). The interaction between cannabinoids and 5-HT neurotransmission is further supported by additional findings (Cheer et al. 1999; Tzavara et al. 2003). Thus, we propose that the increase in 5-HT transmission occurring after MDMA administration could counterbalance the adaptative impairment developed during THC dependence, ameliorating the behavioral manifestations of abstinence. However, a possible pharmacokinetic interaction between MDMA and rimonabant cannot be fully discarded.

In conclusion, this study demonstrates for the first time that acute MDMA administration reduces in a dose-dependent manner the severity of the somatic manifestations of THC abstinence. MDMA affects the changes produced by a chronic administration of THC, but does not directly affect the expression of cannabinoid abstinence precipitated with rimonabant. Furthermore, an increase in 5-HT levels could be related to the behavioral attenuation produced by MDMA on cannabinoid withdrawal syndrome. These results suggest a possible explanation for the associated consumption of cannabis and MDMA in humans. The stimulation of 5-HT neurotransmission elicited by MDMA represents a neurochemical mechanisms to reduce the severity of cannabinoids withdrawal syndrome and represents an interesting direction for future research.

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