Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 384–391
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The CB_1 cannabinoid receptor antagonist AM251 attenuates amphetamine-induced behavioural sensitization while causing monoamine changes in nucleus accumbens and hippocampus

Gunnar Thiemann [a], Vincenzo Di Marzo [b], Areles Molleman [c], Rüdiger U. Hasenöhrl [a,*]

[a] *School of Psychology, Neuroscience Research Unit, University of Hertfordshire, Hatfield, Herts AL10 9AB, UK*
[b] *Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, 80078 (NA), Pozzuoli, Italy*
[c] *School of Life Sciences, University of Hertfordshire, Hatfield, Herts AL10 9AB, UK*

Received 16 September 2007; received in revised form 10 January 2008; accepted 16 January 2008
Available online 26 January 2008

Abstract

Endogenous cannabinoids modulate the activity of dopamine reward pathways and may play a role in the development of behavioural sensitization to psychostimulants. Here, we investigated the effects of the CB_1 cannabinoid receptor antagonist AM251 on amphetamine-induced locomotor sensitization in mice. Furthermore, we measured *post-mortem* monoamine concentrations in nucleus accumbens and hippocampus after termination of the behavioural tests. The results can be summarized as follows: Mice pre-treated with AM251 (3 mg/kg; i.p.) showed less sensitivity to the psychomotor stimulant as well as locomotor sensitizing effects of amphetamine (2 mg/kg; i.p.) resembling previous results obtained with CB_1 receptor-deficient animals. Furthermore, the behavioural effects of AM251 were paralleled by increased dopamine concentration in nucleus accumbens and increased serotonin concentration/turnover rate in hippocampus, respectively. The present data indicate that under normal conditions activation of the CB_1 receptor facilitates those adaptive responses elicited by repeated psychostimulant administration and resulting in sensitization, possibly by reducing dopamine biosynthesis and serotonin turnover in the nucleus accumbens and hippocampus.

Keywords: Basal ganglia; Drug addiction; Craving; CB_1 receptor; Mouse

1. Introduction

There is substantial experimental and clinical evidence suggesting that cannabinoids are drugs of abuse that can activate brain reward substrates, producing dependence, tolerance and abstinence responses (Costa et al., 2000; Gonzalez et al., 2005; Zangen et al., 2006). Certain cannabinoid agonists like WIN 55,212-2 and CP55,940 used in clinically relevant doses as well as the endogenous cannabinoid anandamide are self-administered by rodents and non-human primates in a similar way to amphetamine, cocaine or heroin (Braida et al., 2001; Tanda and Goldberg, 2003; Justinova et al., 2005; Deiana et al., 2007). Similar to psychostimulants and opiates, the habit-forming effects of cannabinoids are associated with a facilitation of the mesolimbic dopaminergic reward pathway leading to increased dopamine levels in the nucleus accumbens (NAcc), which is believed to be responsible for their addictive properties (Van der Stelt and Di Marzo, 2003; Gardner, 2005). Cannabinoids are frequently abused not only alone, but also in combination with other drugs of abuse such as nicotine, alcohol, heroin and amphetamine (Fattore et al., 2007; for review). This raises the question as to whether functional interactions within the mesolimbic reward circuitry influencing susceptibility to drug abuse might also be relevant in the case of the combined use of cannabinoids and other drugs of abuse (Yamamoto et al., 2004).

* Corresponding author. School of Psychology, University of Hertfordshire, College Lane, Hatfield, Herts AL10 9AB, UK. Tel.: +44 1707 284618; fax: +44 1707 285073.
E-mail address: R.U.Hasenoehrl@herts.ac.uk (R.U. Hasenöhrl).

0091-3057/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.01.010

Recent studies specifically addressed this issue by investigating the effect of cannabinoid receptor stimulation/blockade on behavioural response to various indirect dopamine agonist drugs (Pacher et al., 2006; for review). The cannabinoid CB_1 receptor appeared to be not involved in the acute rewarding effects of cocaine, as indicated by the preserved acute cocaine self-administration and cocaine-induced sensitization in CB_1 receptor knockout mice (Cossu et al., 2001) or in mice administered the CB_1 antagonistic compound SR141716A (Lesscher et al., 2005; De Vries et al., 2001; Tanda et al., 2000). Furthermore, the pre-treatment with diverse cannabinoid receptor agonists failed to alter the dopaminergic or behavioural responses to amphetamine questioning the cannabis gateway hypothesis in regard to subsequent amphetamine exposure (Ellgren et al., 2004). However, on the contrary, other studies revealed that endocannabinoid activation of CB_1 receptors mediates the reinforcing effects of cocaine as treatment with SR141716A (rimonabant) decreased the sensitivity of rats to the rewarding effects of cocaine in an intracranial self-stimulation paradigm (Deroche-Gamonet et al., 2001) and the lack of CB_1 receptors impaired cocaine self-administration (Soria et al., 2005). Furthermore, self-administration of ecstasy (MDMA) was reduced in the presence of the cannabinoid agonist CP55,940 and increased after treatment with rimonabant (Braida and Sala, 2002), which, in turn counteracted MDMA-induced place preference (Braida et al., 2005) and potentiated amphetamine-induced hyperlocomotion (Masserano et al., 1999). Moreover, studies from our labs revealed that rimonabant potentiated amphetamine-induced sensitization while, paradoxically, CB_1 receptor-deficient mice showed less sensitivity to the psychomotor stimulant and sensitizing effects of the psychostimulant (Thiemann et al., 2008). The reasons for the conflicting results require further investigation. The main problem with the lack of congruence of the data may lie in the lack of selectivity of the cannabinergic agonists and antagonists used, the known inverse agonistic properties of most cannabinoid antagonists and/or the involvement of different CB_1 and non-CB_1 receptor subtypes in the behavioural effects (Pertwee, 2006).

Starting from this background, the objectives of the present experiment were 2-fold. First, we investigated the effect of the CB_1 receptor antagonist/inverse agonist AM251 on the induction and expression of amphetamine-induced behavioural sensitization in mice. AM251 is structurally related to rimonabant but, compared to the latter, appeared to be more potent and showed higher selectivity for CB_1 receptor (Lan et al., 1999). Furthermore, AM251 suppressed cocaine-primed relapse (Xi et al., 2006) and inhibited food- and methamphetamine-reinforced operant responding (McLaughlin et al., 2003; Vinklerova et al., 2002). Thus, with regard to its pharmacodynamic properties and its known behavioural profile it was held possible that AM251 could reduce or even block the psychomotor stimulant and sensitizing effects of amphetamine, resembling our previous findings obtained with CB_1 receptor-deficient animals (Thiemann et al., 2008). Secondly, we measured monoamine concentrations in the ventral striatum and the hippocampus *post-mortem*, that is, after amphetamine challenge. Monoaminergic neurons are crucially involved in the control of behavioural processes related to reward, addiction and craving (Koob, 1992), and our previous work revealed changes in striatal and hippocampal anandamide and 2-arachidonoylglycerol levels (2-AG) in amphetamine-sensitized mice (Thiemann et al., 2008). With regard to the proposed functional interaction between monoaminergic systems and endocannabinoids (Melis et al., 2004; Van der Stelt and Di Marzo, 2003), we expected to find changes in the concentrations of dopamine (DA), serotonin (5-HT), and their metabolites in animals treated with AM251 alone, and/or in combination with amphetamine.

2. Method

2.1. Animals

The experiments were carried out in accordance with the Animals Scientific Procedures Act 1986 and were approved by the Home Office. Three-month-old male CD1 mice ($N=32$; starting weight 24–35 g; breeder: Charles River, U.K.) were housed 4 per cage and maintained under standard laboratory conditions with a 12D:12L cycle (lights on at 7.30 a.m.). The mice were handled and weighed daily for 7 days; 1 day before the start of the sensitization induction phase, the animals received two injections of physiological saline (5 ml/kg; i.p.) followed by a 60-min habituation trial in the open field to acclimate them to the experimental procedures. All experiments occurred during the 12-h light cycle between 10.00 a.m. and 5.00 p.m.

2.2. Drugs

D-Amphetamine sulphate (Sigma-Aldrich, U.K.) was dissolved in 0.9% saline and injected at doses of 2 mg/kg (induction dose) or 1 mg/kg (challenge dose). The selective CB_1 receptor antagonist AM251 (K_i value = 7.49 nM at CB_1 receptors, 306-fold selective over CB_2 receptors [Gatley et al., 1997; Lan et al., 1999]) was purchased from Tocris (U.K.), dissolved in 0.9% saline containing 2% ethanol and was administered at a dosage of 3 mg/kg. The animals of the control groups received the respective vehicle. All injections were i.p. in a volume of 5.0 ml/kg body weight.

2.3. Apparatus

Horizontal motor activity was measured in square open-field compartments (40 × 40 × 50 cm; black floor and walls) which were set up in a sound-protected experimental chamber adjacent to the animal holding facility. The open-field compartments were placed on top of an under light that provided infrared trans-illumination (880 nm) to a closed circuit video camera (Sanyo, VCB-3572) mounted 2 m above the apparatus. The digitized image of the path taken by each animal was stored and analyzed *post hoc* with a video tracking system (EthoVision; Noldus, The Netherlands) which determined the position of the animal in the open field 5 times per second.

2.4. Design and behavioural procedure

Amphetamine sensitization was induced in mice according to the protocol described by Karper et al. (2002). The experiment consisted of two phases: (a) a sensitization induction phase and (b) a sensitization challenge test phase. Initially, all animals received a 60-min habituation trial in the open field after saline injections administered 30 min and immediately before behavioural testing. Then, the mice were randomly assigned to the following treatment conditions: (i) 5 ml/kg vehicle and 5 ml/kg vehicle (VEH+VEH; $n=8$), (ii) 5 ml/kg vehicle and 2 mg/kg amphetamine (VEH+AMPH; $n=8$), (iii) 3 mg/kg AM251 and 2 mg/kg amphetamine (AM251+AMPH; $n=8$), and (iv) 3 mg/kg AM251 and 5 ml/kg vehicle (AM251+VEH; $n=8$). These groups did not differ in terms of horizontal locomotor activity before start of the induction phase ($F(3,28)=0.01$, $P=0.99$). The animals were injected i.p. with AM251 or corresponding vehicle 30 min before being injected with amphetamine or corresponding vehicle. After being injected, the mice were immediately placed in the open field for a 60-min observation period. All animals received seven daily treatments during the induction phase. Three days after completion of the induction phase, the mice were given one sensitization challenge test. The animals received the corresponding vehicle 30 min before being injected with the challenge dose of amphetamine (1 mg/kg) and locomotor activity was measured again during a 60 min observation period.

2.5. Neurochemical procedures

After the end of behavioural testing, the mice underwent *post-mortem* neurochemical analysis. The animals were sacrificed by decapitation, their brains were quickly removed and the ventral striatum, comprising the nucleus accumbens (NAcc) and hippocampus were dissected out bilaterally on ice. Following dissection, the samples of brain tissue were weighed, placed in plastic tubes containing 0.5 ml of 0.1 M perchloric acid, and then homogenized and centrifuged. The resulting supernatant was filtered through 0.2 µm syringe filters (Chromacol, UK) and the extracts were stored at −70 °C until HPLC-EC analysis. The tissue samples were analyzed for 5-HT, 5-hydroxyindole acetic acid (5-HIAA), dopamine, dihydrophenylacetic acid (DOPAC) and homovanillic acid (HVA) levels. A Waters symmetry C18 column (4.6×150 mm, 3.5 µm) was used. The mobile phase consisted of 50 mM citric acid anhydrous, 20 mM NaCl, 20 mg/l EDTA, 50 mM of 85% H_3PO_4, 100 mg/l Pic B8 (containing water, octane sulfuric acid, methyl alcohol and acetic acid), and 8% MeOH filled up to volume of 5 l with double distilled water. Sodium phosphate monobasic anhydrous (NaH_2PO_4) was used to adjust the pH to 3.2. The solution was filtered using 0.2 µm disc filters (Sigma-Aldrich, UK) and degassed with nitric oxide (NO). The mobile phase flow rate was 0.9 ml/min and a Waters 2465 EC detector was set at 0.7 mV. To quantify the sample peaks each chemical (DA, DOPAC, HVA, 5-HT, 5-HIAA) was compared with external standards that were prepared freshly and injected before and after each sample run.

2.6. Data analysis

The behavioural data were analyzed with single-classification or multivariate analysis of variance, where applicable. To make specific group comparisons, post hoc Tukey tests were performed and $P<0.05$ was used as the criterion for statistical significance. Differences in forebrain monoamine concentrations between groups were assessed by Mann–Whitney's *U*-test (2-tailed) and exact *P*-values were used as a measure of effect. For monoamine–behaviour correlations, Spearman's rank correlation coefficients were calculated (2-tailed).

3. Results

3.1. AM251 decreased amphetamine-induced hyperactivity

An overall statistical analysis was performed upon the results obtained during the 7 days of the sensitization induction phase using group, day, and within-session interval (three 20 min time blocks) each as the three variables. This analysis yielded a significant effect of type of treatment ($F(3,28)=162.59$, $P<0.001$), a significant day group interaction ($F(18,168)=5.11$, $P<0.001$), a significant within-session effect ($F(2,56)=46.07$, $P<0.001$), a significant group interval interaction ($F(6,56)=3.85$, $P=0.003$), and a significant interval day interaction ($F(12,336)=5.92$, $P<0.001$). One-way ANOVAs performed on differences between the VEH+VEH and VEH+AMPH groups for each day yielded *F*-values that were statistically significant at the $P<0.001$ level. Amphetamine injection persistently enhanced locomotion and this effect increased from day 1 to day 7 of induction phase (Fig. 1). Overall, an increase of locomotion was also observed after amphetamine injection

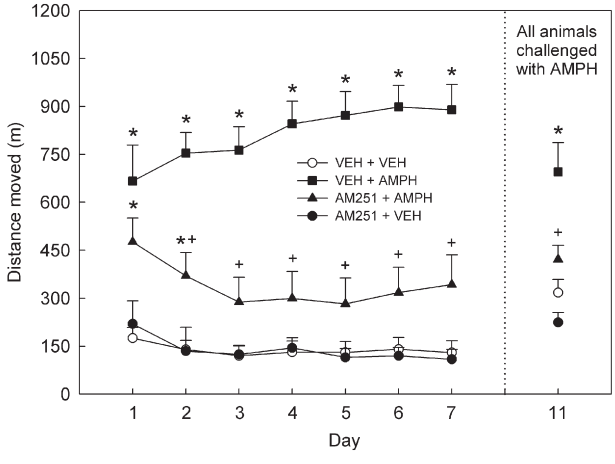

Fig. 1. Effect of the CB_1 receptor antagonist AM251 on amphetamine-induced locomotor activity and sensitization. Mice were injected with AM251 (3 mg/kg, i.p.) or vehicle (VEH; 5 ml/kg) 30 min before being injected with amphetamine (AMPH; 2 mg/kg, i.p.) or VEH on days 1 to 7 (induction phase). All mice were challenged with an injection of amphetamine (1 mg/kg, i.p.) on day 11. Data are presented as mean distance moved (±S.E.M.) during the 60 min period following injections. *Significantly different from VEH+VEH controls; $^{+}$Significantly different from corresponding VEH pre-treated mice. Sample size was 8 per group.

Table 1
Sensitization challenge test: locomotion distance (m) of mice pre-exposed to AM251 and amphetamine injection during sensitization induction phase

Induction treatment	0–20 min	21–40 min	41–60 min
VEH+VEH	119.20±11.03	111.08±18.86	87.25±14.37
VEH+AMPH	289.74±30.09*	218.20±33.64*	187.07±32.93*
AM251+AMPH	206.54±25.39*	134.41±16.65	80.57±10.95^{+}
AM251+VEH	80.44±15.39	70.07±16.46	73.91±11.15

Note. All animals challenged with 1 mg/kg amphetamine. The test period of 60 min was divided into three time blocks of 20 min; values are mean±S.E.M.; *Significantly different from VEH+VEH controls; $^{+}$Significantly different from corresponding VEH pre-exposed mice.

in AM251 pre-treated mice. However, the locomotion response was less prominent and failed statistical significance on induction days 3 to 7. Furthermore, AM251 in combination with amphetamine significantly attenuated the locomotor response produced by the psychostimulant from induction day 2 onwards (VEH+AMPH versus AM251+AMPH), and this effect was most prominent during the second and third 20-min time interval. The pre-treatment with AM251 without amphetamine had no significant effect on locomotor activity on its own.

3.2. AM251 blocked amphetamine-induced locomotion sensitization

Following 7 days of drug treatment, no injections were given to any animals for the next 3 days. To examine the effect of repeated pairings of AM251 with amphetamine for 7 consecutive days on a subsequent amphetamine challenge, the mice received a single injection of amphetamine (1 mg/kg) on day 11. A two-factorial repeated measures ANOVA (treatment × time interval) revealed a significant effect of type of treatment ($F(3,28)=12.83$, $P<0.001$), a significant interval effect ($F(2,56)=30.87$, $P<0.001$), and a significant group interval interaction ($F(6,56)=5.78$, $P<0.001$). Amphetamine pre-treated mice showed an increased locomotor response to the challenge injection, as compared with vehicle pre-treated animals. The effect of amphetamine was significantly attenuated in AM251 pre-treated animals, which, in turn, did not differ from controls. The animals pre-treated with vehicle plus AM251 showed somewhat less locomotion compared with controls; however, the respective *P*-value missed statistical significance. *Post hoc* temporal analysis revealed that during the first 20-min time block, animals pre-treated with amphetamine, irrespective of whether vehicle or AM251 was co-administered during the induction phase, displayed a significantly augmented locomotor response to amphetamine as compared with their VEH pre-treated counterparts. In vehicle pre-treated animals, the amphetamine enhancement of locomotion persisted across time intervals. In contrast, AM251 pre-treated mice did not differ from controls during the second time block and showed less locomotion during the last 20 min interval compared with animals pre-treated with vehicle plus amphetamine, indicative of a CB_1 receptor antagonist induced reduction of the amphetamine-induced behavioural sensitization (Table 1).

3.3. Brain monoamine concentrations

In amphetamine-sensitized mice, 5-HIAA/5-HT ratios were decreased in the hippocampus (−31.0%; $P=0.046$) (Table 2). DA concentrations were increased in the NAcc of animals treated with AM251 in combination with amphetamine (+79%; $P=0.049$) and compared with amphetamine-sensitized mice, these animals showed increased hippocampal 5-HIAA concentrations (+74%; $P=0.022$) and increased 5-HIAA/5-HT ratios (+49%; $P=0.043$). DA and HVA were increased in the NAcc of the animals which were treated with AM251 plus vehicle prior to the amphetamine challenge (DA, +120%; $P=0.036$; HVA, +82%; $P=0.048$).

3.4. Monoamine-behavioural correlations

Correlations between nucleus accumbens and hippocampus monoamine concentrations/ratios and challenge test locomotor activity scores were computed for each of the four

Table 2
Means and SEMs for the ex vivo measurements (in nanograms per milligram) and turnover quotients obtained upon animals sacrificed after the 60-min challenge test trial

Induction treatment	DA	DOPAC	HVA	5-HT	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Ventral striatum								
VEH+VEH	10.36±1.52	2.73±0.55	2.28±0.31	1.51±0.58	0.57±0.19	0.27±0.04	0.23±0.03	0.41±0.08
VEH+AMPH	14.02±2.22	3.46±0.80	2.67±0.17	0.94±0.28	0.43±0.13	0.25±0.03	0.23±0.04	0.40±0.03
AM251+AMPH	18.54±2.34*	2.62±0.75	2.68±0.41	0.82±0.24	0.56±0.07	0.21±0.03	0.24±0.04	0.52±0.10
AM251+VEH	22.80±4.84*	5.43±1.52	4.14±0.75*	1.45±0.36	0.76±0.18	0.22±0.02	0.19±0.03	0.49±0.06
Hippocampus								
VEH+VEH	0.36±0.07	0.09±0.01	*n.d.*	1.81±0.46	1.08±0.32	0.28±0.04	*n.d.*	0.57±0.05
VEH+AMPH	0.49±0.17	0.11±0.24	*n.d.*	1.02±0.31	0.51±0.27	0.33±0.06	*n.d.*	0.39±0.07*
AM251+AMPH	0.27±0.05	0.08±0.01	*n.d.*	2.03±0.46	1.31±0.39^{+}	0.38±0.09	*n.d.*	0.67±0.11^{+}
AM251+VEH	0.27±0.08	0.07±0.01	*n.d.*	1.73±0.74	1.11±0.48	0.37±0.08	*n.d.*	0.63±0.09

Note. All animals challenged with 1 mg/kg amphetamine; *n.d.*, not determined; *$P<0.05$ vs. VEH+VEH controls; $^{+}P\leq0.05$ vs. VEH pre-exposed mice.

Table 3
Spearman rank-order correlation coefficients between monoamine concentrations/turnover quotients and locomotor activity during challenge test

Locomotion/neurochemistry	DA	DOPAC	HVA	5-HT	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Ventral striatum								
VEH+VEH	+0.43	+0.57	+0.43	+0.01	−0.21	+0.24	+0.11	−0.18
VEH+AMPH	+0.33	+0.36	−0.19	+0.19	+0.25	+0.69*	−0.33	+0.11
AM251+AMPH	−0.71*	−0.38	−0.19	−0.02	+0.30	−0.12	+0.81*	+0.90*
AM251+VEH	−0.92**	−0.83*	−0.86*	−0.57	−0.32	−0.33	+0.36	+0.82*
Hippocampus								
VEH+VEH	−0.18	−0.57	*n.d.*	+0.50	+0.29	+0.01	*n.d.*	−0.43
VEH+AMPH	−0.18	−0.11	*n.d.*	+0.36	+0.83*	+0.36	*n.d.*	+0.89*
AM251+AMPH	+0.43	+0.21	*n.d.*	−0.32	−0.50	−0.57	*n.d.*	−0.14
AM251+VEH	−0.54	+0.64	*n.d.*	−0.46	−0.43	−0.18	*n.d.*	−0.14

Note. All animals challenged with 1 mg/kg amphetamine. *$P \leq 0.05$, **$P < 0.01$; *n.d.*, not determined.

treatment groups (Table 3). For amphetamine-sensitized mice (VEH+AMPH), the increased locomotion was accompanied by increased hippocampal 5-HIAA concentrations ($P=0.042$) and enhanced hippocampal 5-HIAA/5-HT ratios ($P=0.019$); a tendency for a positive correlation was obtained between locomotion and accumbal DOPAC/DA ratios ($P=0.072$). In animals which were treated with amphetamine in combination with AM251 during the induction phase (AM251+AMPH) positive monoamine-behavioural correlations were evident for accumbal HVA/DA ($P=0.028$) and 5-HIAA/5-HT ratios ($P=0.037$), while accumbens DA concentrations were negatively correlated with locomotion: the more DA, the lower the locomotor activity ($P=0.047$; Fig. 2). A similar correlation pattern was observed for animals of the AM251 group administered amphetamine on the challenge test day only (AM251+VEH): accumbens 5-HIAA/5-HT ratios were positively correlated with locomotor activity ($P=0.023$), while DA ($P=0.002$), DOPAC ($P=0.010$) as well as HVA ($P=0.014$) concentrations were negatively correlated to behaviour, that is, concentrations were most prominent in those animals showing less locomotor activity during the challenge test trial.

4. Discussion

The present results substantiate that repeated exposure to amphetamine results in behavioural sensitization (Robinson and Becker, 1986; for review). Novel is the finding that the psychomotor stimulant and sensitizing effects of amphetamine could be diminished by joint administration of the CB_1 antagonistic compound AM251 resembling previous findings obtained with CB_1 receptor-deficient mice (Thiemann et al., 2008). AM251, when administered alone, had no effect on locomotion suggesting that there was no significant basal endocannabinoid tone influencing locomotor activity. Furthermore, parameters of monoaminergic activity in nucleus accumbens (NAcc) and hippocampus were affected in sensitized mice and in animals, which were administered the CB_1 antagonist alone or in combination with amphetamine.

The present findings are in line with the outcome of recent studies showing that a gene-targeted disruption of CB_1 receptors can diminish the response to the locomotor-activating and sensitizing effects of amphetamine (Corbille et al., 2007; Thiemann et al., 2008). However, the results with AM251

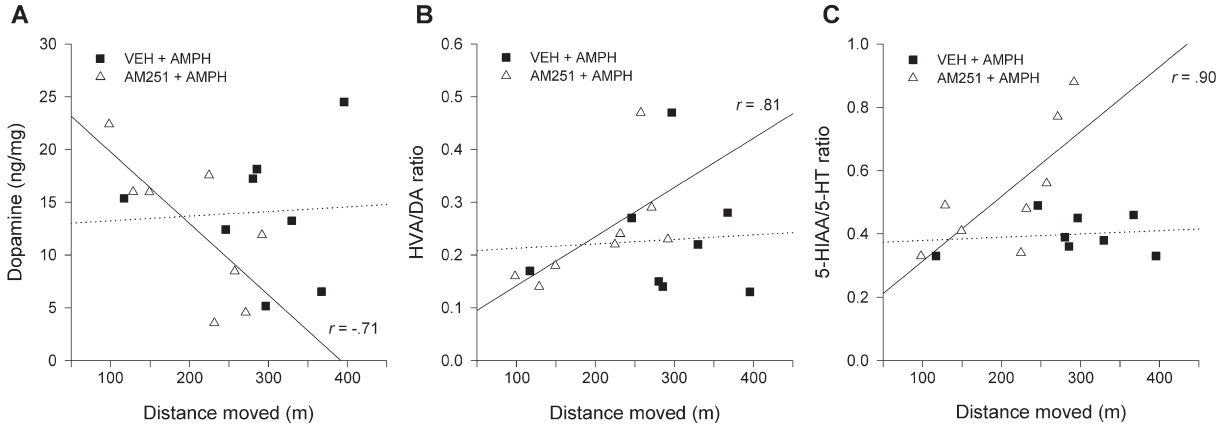

Fig. 2. Examples of significant correlations between locomotor activity and brain monoamine parameters. The distance moved in the open field during challenge test and ventral striatum concentration of dopamine (A) as well as HVA/DA (B) and 5-HIAA/5-HT ratios (C) were correlated for sensitized mice (VEH+AMPH) and for animals pre-treated with AM251 during induction phase (AM251+AMPH). For purposes of presentation, raw values were plotted and linear regression lines fitted to the data (solid, AM251+AMPH; dotted, VEH+AMPH). Spearman rank-order correlation procedures yielded *P*-values <0.05 for the three parameters in the AM251+AMPH group.

reported here are at variance with the effects of rimonabant (SR141716A), which potentiated, rather than blocked amphetamine-induced hyperlocomotion (Masserano et al., 1999) and sensitization (Thiemann et al., 2008), respectively. The fact that AM251, but not rimonabant, inhibited the effect of amphetamine is not entirely surprising with regard to the results of several studies demonstrating that pre-treatment with rimonabant either did not affect or partially blocked the behavioural effects of cannabinoid receptor agonists (Vlachou et al., 2005; for discussion). According to these authors, the discrepancy between the effects of AM251 and rimonabant in counteracting the effects of amphetamine could be explained by the fact that rimonabant either affected a novel cannabinoid receptor, the existence of which has been recently suggested (Di Marzo et al., 2000; Haller et al., 2004) or influenced processes unrelated to the cannabinoid system. Alternatively, rimonabant but not AM251 may act as a partial or inverse agonist at cannabinoid receptors (Pertwee, 2006), although the significance of this action in the observed effects would need further investigation taken into account that both compounds failed to affect behaviour when administered alone. Referring to these assumptions and the present results, our working hypothesis is that amphetamine and AM251 actually share some common sites of action that are probably related to CB_1 receptors in the brain and which are involved in mediating the hyperlocomotor and sensitizing effects of the psychostimulant (see below).

The locomotor-activating effects of amphetamine are thought to depend primarily on its ability to increase dopamine release in the terminal regions of the mesolimbic dopamine system. The ventral tegmental area (VTA) is the somatodendritic region of the mesolimbic dopamine neurons, whose nerve terminals project primarily to the NAcc, the key structure for mediating spontaneous and pharmacologically stimulated locomotor activity. The results of several studies now indicate that amphetamine acts in the VTA to initiate the induction of locomotor sensitization and in the NAcc to promote its expression (Cador et al., 1995; Vezina and Stewart, 1990). Thus, the inhibitory effects of AM251 on the induction of amphetamine sensitization might be theoretically explained by several mechanisms (Gardner, 2005; for review). With regard to the known inhibitory effect of cannabinoid agonists on the release of several neurotransmitters, including glutamate and GABA, it is feasible that AM251 acts in the VTA downstream from the DAergic synapse (Schlicker and Kathmann, 2001). Thus, AM251 might block the disinhibitory action of an endocannabinoid tone on GABAergic neurons, which, *per se*, is insufficient to stimulate motor behaviour, but may play a crucial role in the ability of amphetamine to produce its hyperlocomotor effects. Alternatively, AM251 might also block the modulatory action of endocannabinoids on the excitatory glutamatergic input to the GABA-containing neurons that project from the NAcc to the VTA, which, in concert with presynaptic metabotropic glutamate autoreceptor activation, has been a proposed mechanism of action for the inhibitory effect of AM251 on cocaine-primed relapse (Xi et al., 2006).

However, also direct effects of AM251 on dopamine-dependent substrates at the level of the VTA and the NAcc have to be considered especially in the light of the recent discovery of CB_1 receptors expressed by dopamine neurons in both brain areas (Pickel et al., 2006; Wenger et al., 2003) and the results of *in-vitro* studies with CB_1/CB_2 agonists and CB_1 antagonists demonstrating that the presynaptic modulation of dopamine release in the corpus striatum and the NAcc does not play a role in the extrapyramidal motor and rewarding effects of cannabinoids (Szabo et al., 1999). In this context, the fact that we identified in this study a pharmacological tool, i.e. AM251, which, unlike rimonabant, mimics the effects on amphetamine sensitization previously observed with genetic CB_1 receptor blockade (Thiemann et al., 2008), allowed us to perform analyses of monoamine levels and turnover in *post-mortem* brains of animals treated with and subsequently challenged with amphetamine in the presence or absence of this CB_1 receptor antagonist. The results of these neurochemical analyses are not biased by possible adaptive phenomena occurring in CB_1 knockout mice and add important information to the behavioural results described in the present and the previous study (Thiemann et al., 2008). Both groups of animals treated with AM251 either in combination with amphetamine or vehicle controls exhibited increased dopamine levels in the nucleus accumbens after amphetamine challenge and the accumbens dopamine concentration was negatively correlated with locomotor activity, that is, the more dopamine, the less locomotor sensitization. Furthermore, several measures of serotonin activity were affected in AM251 treated animals. In a recent study, peripheral administration of AM251 failed to produce significant effects on extracellular NAcc dopamine, albeit a tendency for an increase was observed (Xi et al., 2006). Furthermore, we found that amphetamine sensitization is related to decreased anandamide and 2-AG levels in the ventral striatum, comprising nucleus accumbens (Thiemann et al., 2008). Based on the results of only these few studies, detailed considerations regarding the mode of action of AM251 as well as the relationship between CB_1 receptive sites, dopamine, and amphetamine sensitization would be premature. However, altogether, the data provide further supportive evidence for a reciprocally acting regulatory mechanism in the control of sensorimotor and neuroadaptive processes involving CB_1 receptors and striatal dopamine (Giuffrida et al., 1999; Gorriti et al., 1999).

The functional significance of the observed action of AM251 on dopamine and serotonin neurochemistry in terms of behavioural sensitization is open to question. In general, behavioural sensitization is used as an animal model for studying the development of craving in addicts and psychosis that arises from repeated exposure to psychostimulants (Robinson and Becker, 1986). Furthermore, it is known that withdrawal of a drug of abuse (in the present study the 3-day period between induction and amphetamine challenge phase) produces craving, which is accompanied by decreased dopaminergic (Diana et al., 1998) and serotonergic receptor function (Przegalinski et al., 2003; for review). Therefore, it might be taken into consideration that inhibition of endocannabinoid receptive sites by AM251 could reduce expression of amphetamine sensitization by lowering craving during withdrawal due to its stimulatory effects on central dopaminergic and serotonergic activity. Such 'anti-craving' effects of AM251 have also been hypothesized to play a

paramount role for the inhibitory action of the CB_1 antagonist on methamphetamine-produced self-administration (Vinklerova et al., 2002).

5. Conclusion

The results of the present study revealed that the acute blockade of CB_1 receptors by AM251 has profound effects on amphetamine sensitization. Most strikingly, besides the inhibition of the hyperlocomotor stimulant effect of the psychostimulant, a clear attenuation of the expression of amphetamine sensitization by AM251 was observed, which was accompanied by changes in accumbens and hippocampus dopamine and serotonin concentration/turnover, respectively. With regard to the paucity of effective medications to treat psychostimulant addiction, the present findings suggest that CB_1 receptor antagonists, especially AM251, may be promising as anti-craving drugs in relapse prevention for (amphetamine) addiction.

References

Braida D, Sala M. Role of the endocannabinoid system in MDMA intracerebral self-administration in rats. Br J Pharmacol 2002;136:1089–92.

Braida D, Pozzi M, Parolaro D, Sala M. Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol 2001;413:227–34.

Braida D, Iosue S, Pegorini S, Sala M. 3,4 Methylenedioxymethamphetamine-induced conditioned place preference (CPP) is mediated by endocannabinoid system. Pharmacol Res 2005;51:177–82.

Cador M, Bjijou Y, Stinus L. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience 1995;65:385–95.

Corbille AG, Valjent E, Marsicano G, Ledent C, Lutz B, Herve D, et al. Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. J Neurosci 2007;27:6937–47.

Cossu G, Ledent C, Fattore L, Imperato A, Böhme GA, Parmentier M, et al. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav Brain Res 2001;118:61–5.

Costa B, Giagnoni G, Colleoni M. Precipitated and spontaneous withdrawal in rats tolerant to anandamide. Psychopharmacology 2000;149:121–8.

Deiana S, Fattore L, Spano MS, Cossu G, Porcu E, Fadda P, et al. Strain and schedule-dependent differences in the acquisition, maintenance and extinction of intravenous cannabinoid self-administration in rats. Neuropharmacology 2007;52:646–54.

Deroche-Gamonet V, Le Moal M, Piazza PV, Soubrie P. SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. Psychopharmacology 2001;157:254–9.

De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, et al. A cannabinoid mechanism of relapse to cocaine seeking. Nat Med 2001;7:1151–4.

Diana M, Melis M, Muntoni AL, Gessa GL. Mesolimbic dopaminergic decline after cannabinoid withdrawal. Proc Natl Acad Sci USA 1998;18:10269–73.

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, et al. Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000;75:2434–44.

Ellgren M, Hurd YL, Franck J. Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. Eur J Pharmacol 2004;497:205–13.

Fattore L, Spano MS, Deiana S, Melis V, Cossu G, Fadda P, et al. An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. Brain Res Brain Res Rev 2007;53:1–16.

Gardner EL. Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav 2005;81:263–84.

Gatley SJ, Lan R, Pyatt B, Gifford AN, Volkow ND, Makriyannis A. Binding of the non-classical cannabinoid CP 55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci 1997;61:PL 191–7.

Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 1999;2:358–63.

Gonzalez S, Cebeira M, Fernandez-Ruiz J. Cannabinoid tolerance and dependence: a review of studies in laboratory animals. Pharmacol Biochem Behav 2005;81:300–18.

Gorriti MA, Rodriguez de Fonseca F, Navarro M, Palomo T. Chronic (−)-Δ9-tetrahydrocannabinol treatment induces sensitization to the psychomotor effects of amphetamine in rats. Eur J Pharmacol 1999;365:133–42.

Haller J, Varga B, Ledent C, Freund TF. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav Pharmacol 2004;15:299–304.

Justinova Z, Solinas M, Tanda G, Redhi GH, Goldberg SR. The endogenous cannabinoid anandamide and its synthetic analog *R*(+)-methanandamide are intravenously self-administered by squirrel monkeys. J Neurosci 2005;25:5645–50.

Karper PE, De la Rosa H, Newman ER, Krall CM, Nazarian A, McDougall SA, et al. Role of D1-like receptors in amphetamine-induced behavioral sensitization: a study using D1A receptor knockout mice. Psychopharmacology 2002;159:407–14.

Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.

Lan R, Liu Q, Fan P, Lin S, Fernando SR, McCallion D, et al. Structure–activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. J Med Chem 1999;42:769–76.

Lesscher HM, Hoogveld E, Burbach PH, van Ree JM, Gerrits MA. Endogenous cannabinoids are not involved in cocaine reinforcement and development of cocaine-induced behavioral sensitization. Eur Neuropsychopharmacol 2005;15:31–7.

Masserano JM, Karoum F, Wyatt RJ. SR 141716A, a CB1 cannabinoid receptor antagonist, potentiates the locomotor stimulant effects of amphetamine and apomorphine. Behav Pharmacol 1999;10:429–32.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, et al. The cannabinoid CB1 antagonists SR141716A and AM251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol 2003;14:583–8.

Melis M, Pisitis M, Perra S, Muntoni AL, Pillolla G, Gessa GL. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 2004;24:53–62.

Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58:389–462.

Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond) 2006;30 Suppl 1:S13–8.

Pickel VM, Chan J, Kearn CS, Mackie K. Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. J Comp Neurol 2006;495:299–313.

Przegalinski E, Czepiel K, Nowak E, Dlaboga D, Filip M. Withdrawal from chronic cocaine up-regulates 5-HT1B receptors in the rat brain. Neurosci Lett 2003;351:169–72.

Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 1986;396:157–98.

Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 2001;22:565–72.

Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M, et al. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. Neuropsychopharmacology 2005;30:1670–80.

Szabo B, Muller T, Koch H. Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens in vitro. J Neurochem 1999;73:1084–9.

Tanda G, Goldberg SR. Cannabinoids: reward, dependence, and underlying neurochemical mechanisms-a review of recent preclinical data. Psychopharmacology 2003;169:115–34.

Tanda G, Munzar P, Goldberg SR. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci 2000;3:1073–4.

Thiemann G, van der Stelt M, Petrosino S, Molleman A, Di Marzo V, Hasenohrl RU. The role of the CB1 cannabinoid receptor and its endogenous ligands, anandamide and 2-AG, in amphetamine-induced behavioural sensitization. Behav Brain Res 2008;187:289–96.

Van der Stelt M, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. Eur J Pharmacol 2003;480:133–50.

Vezina P, Stewart J. Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Res 1990;516:99–106.

Vinklerova J, Novakova J, Sulcova A. Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM251. J Psychopharmacol 2002;16:139–43.

Vlachou S, Nomikos GG, Panagis G. CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. Psychopharmacology 2005;179:498–508.

Wenger T, Moldrich G, Furst S. Neuromorphological background of cannabis addiction. Brain Res Bull 2003;61:125–8.

Xi ZX, Gilbert JG, Peng XQ, Pak AC, Li X, Gardner EL. Cannabinoid CB1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. J Neurosci 2006;26:8531–6.

Yamamoto T, Anggadiredja K, Hiranita T. New perspectives in the studies on endocannabinoid and cannabis: a role for the endocannabinoid-arachidonic acid pathway in drug reward and long-lasting relapse to drug taking. J Pharmacol Sci 2004;96:382–8.

Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA. Two brain sites for cannabinoid reward. J Neurosci 2006;26:4901–7.