Cannabinoids prevent the acute hyperthermia and partially protect against the 5-HT depleting effects of MDMA ("Ecstasy") in rats

Kirsten C. Morley a, Kong M. Li b, Glenn E. Hunt c, Paul E. Mallet d, Iain S. McGregor a,∗

a School of Psychology, University of Sydney, Sydney, NSW 2006, Australia
b Department of Pharmacology, University of Sydney, Sydney, NSW 2006, Australia
c Department of Psychological Medicine, University of Sydney, Concord Hospital, Sydney, NSW 2139, Australia
d School of Psychology, University of New England, Armidale, NSW 2351, Australia

Received 21 August 2003; received in revised form 15 December 2003; accepted 7 January 2004

Abstract

Cannabinoid–MDMA interactions were examined in male Wistar rats. MDMA (4 2 5 mg/kg or 2 10 mg/kg over 4 h on each of 2 days) was administered with or without Δ9-tetrahydrocannabinol (THC) (4 2 2.5 mg/kg), the synthetic cannabinoid receptor agonist CP 55,940 (2 2 0.1 or 0.2 mg/kg) or the cannabinoid receptor antagonist SR 141716 (2 2 5 mg/kg). Co-administered Δ9-THC and CP 55,940 but not SR 141716 prevented MDMA-induced hyperthermia, causing a powerful hypothermia. Co-administered Δ9-THC, CP 55,940 and SR 141716 all tended to decrease MDMA-induced hyperactivity. Co-administered Δ9-THC provided protection against the long-term increases in anxiety seen in the emergence test, but not the social interaction test, 6 weeks after MDMA treatment. Co-administered Δ9-THC and CP 55,940, but not SR 141716, partly prevented the long-term 5-HT and 5-HIAA depletion caused by MDMA in various brain regions. SR 141716 administered with CP 55,940 and MDMA prevented the hypothermic response to the CP 55,940/MDMA combination but did not alter the CP 55,940 attenuation of MDMA-induced 5-HT depletion. These results suggest a partial protective effect of co-administered cannabinoid receptor agonists on MDMA-induced 5-HT depletion and long-term anxiety. This action appears to operate independently of cannabinoid CB1 receptors.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: MDMA; Ecstasy; Cannabinoid; Hyperthermia; Hypothermia; Neurotoxicity

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") is a widely used illicit drug. The possible adverse effects of MDMA use are a matter of some considerable concern. In laboratory animals, MDMA causes 5-HT nerve terminal degeneration with a concomitant reduction in brain 5-HT content (for reviews see Ricaurte et al., 2000; Green et al., 2003). Human MDMA users also exhibit several effects consistent with 5-HT depletion (Boot et al., 2000; McCann et al., 1998; Parrott, 2001; Reneman et al., 2001) and this may be linked to a number of adverse psychological effects including anxiety, depression and impaired cognition (Morgan, 2000; Parrott, 2001). However, a specific causal relationship between MDMA use and psychological dysfunction is difficult to demonstrate. This is because of the polydrug use that is typical of human MDMA users and the possibility of pre-existing psychopathology in users (Cole et al., 2002; Croft et al., 2001).

Brief exposure to MDMA in rats causes a long-lasting syndrome consisting of greater anxiety-like behaviours, impaired memory and depressive-like symptoms (Bull et al., 2003; Fone et al., 2002; Gurtman et al., 2002; McGregor et al., 2003a,b; Morley et al., 2001). Such effects are consistent with human reports and may reflect MDMA-induced 5-HT depletion and/or alterations in
the density of 5-HT receptors and transporters (McGregor et al., 2003a,b).

The neurotoxic effects of MDMA are modulated by dose, frequency and route of administration (O'Shea et al., 1998; Ricaurte et al., 2000) as well as the hyperthermic action of the drug (Malberg et al., 1996; Malberg and Seiden, 1998; but see also McGregor et al., 2003b). Interventions that prevent MDMA-induced hyperthermia protect against the neurotoxic effects of the drug (Colado et al., 1998; Malberg et al., 1996; O'Shea et al., 2001). Drugs with antioxidant properties also attenuate the neurotoxic effects of MDMA (Colado and Green, 1995; Colado et al., 1997; Shankaran et al., 2001).

The drug most commonly taken with MDMA in human users is cannabis. More than 90% of ecstasy users take cannabis regularly (Siliquini et al., 2001; Topp et al., 1999). Cannabis is frequently used to mitigate the MDMA “comedown” (Winstock et al., 2001) but is also commonly used before and during dance parties at which MDMA is taken (Boys et al., 1997).

The primary psychoactive constituent of cannabis, Δ⁹-tetrahydrocannabinol (THC), and synthetic cannabinoid receptor agonists such as CP 55,940, cause hypothermia (Arnold et al., 2001; Fennessy and Taylor, 1978; Pryor et al., 1978). Recent research also indicates that these cannabinoids have antioxidant properties (Hampson et al., 1998; Leker et al., 1999; Marsicano et al., 2002; Nagayama et al., 1999; Shohami and Mechoulam, 2000). This suggests that co-administered cannabinoids might modulate the long-term neurotoxic properties of MDMA either through a hypothermic action, an antioxidant action, or both.

The present study addressed this possibility. Firstly, the effects of co-administered THC on the acute hyperthermia and long-term behavioural and neural effects of MDMA were investigated. Then the possible neuroprotective effects arising from the co-administration of the synthetic cannabinoid CP 55,940 and the cannabinoid CB₁ receptor antagonist SR 141716 were examined.

2. Materials and methods

2.1. Animals

Inbred male albino Wistar rats were used in all experiments. They were housed in large plastic tubs in groups of 6–8 per cage in a temperature-controlled environment (mean 22°C) with food and water freely available. A 12 h reversed light cycle was in operation with lights off at 8:30 am. Testing took place during the dark cycle. All experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996. Every effort was made to minimize the number of animals used and their suffering. Approval for the experiments was obtained from the University of Sydney Animal Ethics Committee.

2.2. Drugs

(+/-)3,4-Methylenedioxymethamphetamine hydrochloride (MDMA) and Δ⁹-tetrahydrocannabinol (THC) were obtained from the Australian Government Analytical Laboratories (Pymble, NSW). The MDMA was dissolved in 0.9% saline. THC was dissolved in absolute ethanol and Tween 80 was added to this solution. The ethanol was evaporated under a stream of nitrogen gas and the resulting solution was diluted with 0.9% saline to give a final dilution of 1:19 (Tweak 80/saline). CP 55,940 and SR 141716 were obtained from Tocris (Bristol, UK) and Sanofi (Montpelier, France), respectively. CP 55,940 was dissolved in absolute ethanol and Tween 80 was added to this solution. The resulting solution was diluted with 0.9% saline to give a final dilution of 1:1:18 (ethanol/Tween 80/saline). SR 141716 was mixed with Tween 80 and 0.9% saline was added to this solution to give a final dilution of 1:1:1:19 (Tweak 80/saline). All drugs were injected intraperitoneally at a volume of 1 ml/kg body weight.

2.3. Locomotor activity measurement

Locomotor activity during acute drug administration was measured in standard operant chambers (30 × 50 × 25.5 cm) fitted with passive infrared detectors as described previously (Morley et al., 2001). Infrared detectors of greater sensitivity were used in Experiments 2 and 3, giving higher locomotor activity counts than in Experiment 1. Immediately following drug administration, individual rats were placed in the test chambers for 4 h in a room where the ambient temperature was set between 26 and 28°C. Every 60 min the rats were taken out of the locomotor activity testing cages for measurement of body temperature and (depending upon the experiment) to receive their next drug treatment. At the end of each 4 h session the rats were returned to their home cages in the colony room at a temperature of 22°C.

2.4. Body temperature measurement

Body temperature was measured at baseline, immediately prior to the first drug injection, and then every hour during drug testing. This was done using a Braun Thermoscan Instant Thermometer (IRT 1020) inserted into the ear of the rat as described previously (Gurtman et al., 2002; Morley et al., 2001).
2.5. \textit{Emergence test}

The emergence apparatus was as described previously (Gurtman et al., 2002; Morley et al., 2001), consisting of a white wooden walled rectangular arena (96 × 100 × 40 cm) illuminated with red light (40 W) and containing a black wooden hide box (24 × 40 × 15 cm). The rats were placed in the hide box and their behaviour was recorded for 5 min by a miniature video camera. The apparatus was thoroughly wiped down with 10% ethanol in between sessions. An observer ‘blind’ to the group allocation subsequently scored latency to emerge from the hide box, emergence frequency, duration of time spent in the open field, risk assessment and number of rears. Scoring was performed using the ODLog scoring software package from Macropod Software (www.macropodsoftware.com).

2.6. \textit{Social interaction test}

The social interaction test was as described previously (Morley et al., 2001). Testing was conducted in low light conditions with an unfamiliar conspecific. This configuration allows detection of both anxiolytic and anxiogenic effects of drugs (File, 1980; Gurtman et al., 2002; Morley et al., 2001; Morley and McGregor, 2000). The rats were placed in a square clear Perspex box (52 × 52 × 40 cm), dimly lit with red light (40 W) with a miniature video camera positioned above. Pairs of rats of similar body weight from the same treatment groups but from different home cages were tested together for 10 min. The box was wiped down with a 10% ethanol solution between each test session. The number and duration of social interactions were later scored according to the method described by File (1980) by a ‘blind’ observer using ODLog software.

2.7. \textit{Neurochemical analysis}

At a designated interval following drug treatment, rats were decapitated using a guillotine, and their brains rapidly removed for neurochemical analysis. Three regions of interest were manually dissected out over dry ice as described previously (McGregor et al., 2003a,b). Samples from the prefrontal cortex, hippocampus and amygdala were individually placed in centrifuge tubes and were stored in a freezer at −80 °C until assayed. Tissue samples were weighed and then homogenized with a 500 μl ice-cold solution of 0.2 M perchloric acid containing 0.1% cysteine and 100 nmol/l of internal standard 5-hydroxy- N-methyltryptamine (5-HMeT). The homogenate was centrifuged at 15,000 g for 10 min at 4 °C and a 20 μl aliquot of the resulting supernatant fluid was then analysed for biogenic amines by high performance liquid chromatography (HPLC) with electro-chemical detection as described previously (McGregor et al., 2003a,b). The GBC HPLC system (Melbourne, Australia) and protocol was as described by Gurtman et al. (2002). For Experiments 2 and 3, details for the HPLC protocol were as for Experiment 1 except that the HPLC system consisted of a Shimadzu ADVP module (Kyoto, Japan) equipped with SIL-10 auto-injector with sample cooler and LC-10 on-line vacuum degassing solvent delivery unit. Chromatographic control, data collection and processing were carried out using Shimadzu Class VP data software.

2.8. \textit{Procedure}

2.8.1. Experiment 1: the effect of co-administered THC on MDMA-induced hyperactivity, hyperthermia, anxiety and 5-HT depletion

Rats (n= 52), aged between 95 and 105 days, weighing an average of 410 g at the start of treatment were used. They were randomly assigned to the following groups (n= 13 per group): vehicle, MDMA, THC, and MDMA/THC. A MDMA dose regime of 5 mg/kg per hour for 4 h on each of two consecutive days (2 × 4 × 5 mg/kg) was chosen as we have previously shown that this regime causes reliable long-term 5-HT depletion and long-term increases in anxiety (Gurtman et al., 2002; Morley et al., 2001). Rats in the THC group were given repeated doses of 2.5 mg/kg THC using the same regimen (2 × 4 × 2.5 mg/kg). Rats in the MDMA/THC dose treatment group were given 5 mg/kg MDMA per hour for 4 h on each of two consecutive days (2 × 4 × 5 mg/kg) concomitantly with 2.5 mg/kg THC using the same regimen (2 × 4 × 2.5 mg/kg). See Table 1 for summary.

Testing on the emergence test occurred approximately 6 weeks following drug treatment, with all rats tested on a single day in the drug-free state. The social interaction test was conducted on the day after the emergence test. At 7 weeks following drug treatment, 40 of the 52 rats used in the study (n= 10 per group) were randomly selected, decapitated and their brains rapidly removed for neurochemical analysis.

2.8.2. Experiment 2: the effect of co-administered CP 55,940 on MDMA-induced hyperactivity, hyperthermia and 5-HT depletion

Rats (n= 40) aged between 70 and 80 days were used, weighing an average of 325 g at the start of treatment. They were randomly assigned to the following five groups (n= 8 per group): vehicle, MDMA, MDMA/CP0.1, MDMA/CP0.2 and MDMA/CP0.2/SR. The treatment regimes for each of these groups are shown in Table 1. All of the rats received three concomitant injections every 2 h for 4 h on each of two consecutive days. Locomotor activity and body tem-
perature were measured every hour during drug administration. At 14 days following drug treatment, all rats were decapitated using a guillotine, and their brains rapidly removed for neurochemical analysis.

2.8.3. Experiment 3: the effect of co-administered SR 141716 on MDMA-induced hyperactivity, hyperthermia and 5-HT depletion

Rats (n = 32) aged between 70 and 80 days were used, with an average weight of 336 g at the start of testing. They were randomly assigned to four groups (n = 8 per group): vehicle, MDMA, SR and MDMA/SR. The treatment regimes for each of these groups are shown in Table 1. All rats received two concomitant injections every 2 h for 4 h on each of two consecutive days. Vehicle group rats received equivalent injections of saline and vehicle solutions. Locomotor activity and core body temperature were recorded every hour. Fourteen days following drug treatment, all rats were decapitated and their brains rapidly removed for neurochemical analysis.

2.9. Statistical analysis

All data were analysed using one-way analysis of variance (ANOVA) followed by Student Newman–Keuls post-hoc tests. A probability level of 0.05 was used for all statistical comparisons.

Body temperature changes were analysed as follows. For the five temperature readings (baseline and hours 1–4), the peak temperature deviation from baseline during the 4 h session was calculated in both positive and negative direction for each rat (i.e. highest and lowest body temperature expressed as change from baseline). This maximal change in both directions was then compared across groups using one-way ANOVA followed by Student Newman–Keuls post-hoc tests.

For clarity of exposition HPLC values are presented as a percentage change from the control group mean in each experiment. However, all statistics were performed on untransformed data expressed as ng/g of tissue.

3. Results

3.1. Experiment 1: the effect of co-administered THC on MDMA-induced hyperactivity, hyperthermia, anxiety and 5-HT depletion

3.1.1. Body temperature

Body temperature changes on the 2 days of drug administration are depicted in Fig. 1. For day 1, ANOVA revealed significant differences between groups for highest \(F(3,48) = 12.10, P < 0.0001\) and lowest \(F(3,48) = 6.73, P < 0.001\) body temperature recorded (expressed as change from pre-drug baseline). Similar results were obtained on day 2 of drug treatment \(F(3,48) = 16.43, P < 0.0001\) and \(F(3,48) = 23.69, P < 0.0001\), respectively. Post-hoc analyses (Fig. 1) revealed a significant hyperthermic effect of MDMA and significant hypothermic effects of the MDMA/THC combination for both days of testing. The THC group also displayed significant hypothermia relative to the vehicle group but not to the same extent as the MDMA/THC group.

3.1.2. Locomotor activity

Locomotor activity counts on the two drug administration days are depicted in Fig. 1. ANOVA revealed a significant overall group effect on day 1 \(F(3,48) = 12.52, P < 0.0001\) and day 2 \(F(3,48) = 39.78, P < 0.0001\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Frequency a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>VEH</td>
<td>SAL + VEH</td>
</tr>
<tr>
<td></td>
<td>THC</td>
<td>SAL + THC (2.5 mg/kg)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>MDMA (5 mg/kg) + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA/THC</td>
<td>MDMA (5 mg/kg) + THC (2.5 mg/kg)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>VEH</td>
<td>SAL + VEH + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>MDMA (10 mg/kg) + VEH + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA/CP0.1</td>
<td>MDMA (10 mg/kg) + CP (0.1 mg/kg) + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA/CP0.2</td>
<td>MDMA (10 mg/kg) + CP (0.2 mg/kg) + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA/CP0.2/SR</td>
<td>MDMA (10 mg/kg) + CP (0.2 mg/kg) + SR (5 mg/kg)</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>VEH</td>
<td>SAL + VEH</td>
</tr>
<tr>
<td></td>
<td>SR</td>
<td>SAL + SR (5 mg/kg)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>MDMA (10 mg/kg) + VEH</td>
</tr>
<tr>
<td></td>
<td>MDMA/SR</td>
<td>MDMA (10 mg/kg) + SR (5 mg/kg)</td>
</tr>
</tbody>
</table>

SAL, saline; VEH, vehicle; SR, SR 141716; CP, CP 55,940.

a Frequency refers to doses x days. Doses were always administered equally spaced over a 4 h period on each day so 4 x 2 refers to four doses over 4 h (i.e. one per hour) on each of two consecutive days.

Table 1
Summary of drug treatments used in Experiments 1, 2 and 3
Post-hoc analyses (Fig. 1) revealed significantly greater activity in the MDMA group and MDMA/THC groups relative to both the vehicle and THC groups on both days of testing. The MDMA group also showed significantly greater activity than the MDMA/THC group on day 2 of testing.

3.1.3. Emergence test

The results for the emergence test are shown in Table 2. One-way ANOVA revealed an overall significant group effect on emergence latency ($F(3, 48) = 10.58$, $P < 0.01$), emergence frequency ($F(3, 48) = 3.85$, $P < 0.05$), open field time ($F(3, 48) = 6.62$, $P < 0.01$), risk assessment ($F(3, 48) = 2.87$, $P < 0.05$) and duration.

Data represent mean (SEM).

Maximum emergence latency = 300 s due to 5 min test.

* $P < 0.05$ relative to vehicle group.

$\# P < 0.05$ relative to THC group.

$^c P < 0.05$ relative to MDMA/THC group, Student Newman–Keuls post-hoc tests.

---

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Emergence latency (s)</th>
<th>Time in open field (s)</th>
<th>Emergence frequency (n)</th>
<th>Rearing (s)</th>
<th>Risk assessment (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>58.84 (10.32)</td>
<td>117.02 (10.93)</td>
<td>4.19 (0.40)</td>
<td>11.19 (1.08)</td>
<td>22.85 (2.54)</td>
</tr>
<tr>
<td>THC</td>
<td>96.73 (18.07)</td>
<td>130.34 (20.85)</td>
<td>2.81 (0.45)</td>
<td>12.63 (2.01)</td>
<td>39.66 (9.52)</td>
</tr>
<tr>
<td>MDMA</td>
<td>219.19 (27.37)$^{a,b,c}$</td>
<td>51.70 (23.18)$^{a,b,c}$</td>
<td>1.85 (0.48)$^{a,b,c}$</td>
<td>18.92 (2.39)$^{a,b,c}$</td>
<td>55.10 (8.49)$^{a}$</td>
</tr>
<tr>
<td>MDMA/THC</td>
<td>116.43 (25.62)</td>
<td>112.06 (20.13)</td>
<td>3.64 (0.65)</td>
<td>13.93 (1.47)</td>
<td>42.57 (9.02)</td>
</tr>
</tbody>
</table>

---

18.50, $P < 0.0001$. Post-hoc analyses (Fig. 1) revealed significantly greater activity in the MDMA group and MDMA/THC groups relative to both the vehicle and THC groups on both days of testing. The MDMA group also showed significantly greater activity than the MDMA/THC group on day 2 of testing.
of rearing \( F(3, 48) = 3.33, P<0.05 \). Post-hoc analyses (Table 2) revealed that the MDMA group showed greater anxiety on a number of these measures compared to the vehicle, THC and MDMA/THC groups.

### 3.1.4. Social interaction test

The results for the social interaction test are shown in Table 3. One-way ANOVA revealed a significant overall group effect for the duration of social interaction \( F(3, 20) = 6.78, P<0.05 \) and the number of social interactions \( F(3, 20) = 5.29, P<0.01 \). Post-hoc analyses revealed that both the MDMA and MDMA/THC groups displayed significantly less duration of social interaction and number of interactions than the vehicle or THC groups.

### 3.1.5. Neurochemical analysis

The HPLC results are presented in Table 4. One-way ANOVA revealed a significant overall group effect for 5-HT in the prefrontal cortex \( F(3, 36) = 3.40, P<0.05 \), hippocampus \( F(3, 36) = 6.12, P<0.01 \) and amygdala \( F(3, 36) = 3.00, P<0.05 \). There were also significant overall group effects for 5-HIAA in the prefrontal cortex \( F(3, 36) = 7.07, P<0.001 \), hippocampus \( F(3, 36) = 4.54, P<0.01 \) and amygdala \( F(3, 36) = 2.88, P<0.05 \). There were no significant differences between groups in dopamine concentrations in any of the brain regions examined \( F(3, <1.2) \) (data not shown).

Post-hoc analyses revealed significantly lower 5-HT and 5-HIAA levels in the MDMA group relative to the vehicle and THC groups in all brain regions examined. The MDMA/THC group displayed significantly lower 5-HT levels than the vehicle and the THC groups in the hippocampus only. The MDMA group had significantly lower 5-HIAA in the prefrontal cortex than the MDMA/THC group.

### 3.2. Experiment 2: the effect of co-administered CP 55,940 on MDMA-induced hyperactivity, hyperthermia and 5-HT depletion

#### 3.2.1. Body temperature

Body temperature changes on the 2 days of drug administration are depicted in Fig. 2. For day 1, ANOVA revealed significant differences between groups for highest \( F(4, 35) = 26.82, P < 0.0001 \) and lowest \( F(4, 35) = 9.96, P < 0.0001 \) body temperature recorded (expressed as change from pre-drug baseline). A similar pattern of results was evident on day 2: \( F(4, 35) = 10.82, P < 0.0001 \) and \( F(4, 35) = 19.19, P < 0.0001 \), respectively. Post-hoc analyses (Fig. 2) revealed a significant hyperthermia in the MDMA, MDMA/CP0.1 and the MDMA/CP0.2/SR groups on both days of testing. In contrast, there was a significant hypothermia in the MDMA/CP0.2 group.

#### 3.2.2. Locomotor activity

Locomotor activity on the two drug administration days is depicted in Fig. 2. On day 1 and day 2, ANOVA revealed a significant overall group effect \( F(4, 35) = 9.82, P < 0.0001 \) and \( F(4, 35) = 6.84, P < 0.001 \). Post-hoc analyses (Fig. 2) revealed that there was significantly higher activity in the MDMA group relative to all other groups on day 1. On day 2, the MDMA group had greater activity than the vehicle, MDMA/CP0.1 and the MDMA/CP0.2 groups while the MDMA/CP0.2/SR group showed significantly greater activity than the vehicle and MDMA/CP0.2 groups.

---

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Time in social interaction (s)</th>
<th>Number of interactions (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>109.59 (5.55)</td>
<td>56.71 (3.97)</td>
</tr>
<tr>
<td>THC</td>
<td>116.59 (9.10)</td>
<td>54.13 (3.05)</td>
</tr>
<tr>
<td>MDMA</td>
<td>83.48 (6.74)(^{a,b})</td>
<td>42.92 (2.82)(^{a,b})</td>
</tr>
<tr>
<td>MDMA/THC</td>
<td>75.53 (9.18)(^{a,b})</td>
<td>42.25 (2.49)(^{a,b})</td>
</tr>
</tbody>
</table>

Data represent mean (SEM).

\(^{a}\) \( P<0.01 \) relative to vehicle group.

\(^{b}\) \( P<0.01 \) relative to THC group, Student Newman–Keuls post-hoc tests.

Table 4

<table>
<thead>
<tr>
<th>Region</th>
<th>Treatment</th>
<th>5-HT (n= 10 per group)</th>
<th>5-HIAA (n= 10 per group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal</td>
<td>THC</td>
<td>95.64 (10.47)</td>
<td>98.13 (7.37)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>61.33 (3.81)(^{a,b})</td>
<td>57.60 (4.35)(^{a,b})</td>
</tr>
<tr>
<td></td>
<td>MDMA/THC</td>
<td>79.92 (8.94)</td>
<td>79.30 (8.09)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>THC</td>
<td>100.60 (12.06)</td>
<td>103.45 (16.40)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>52.80 (5.83)(^{a,b})</td>
<td>54.92 (6.11)(^{a,b})</td>
</tr>
<tr>
<td></td>
<td>MDMA/THC</td>
<td>65.37 (7.99)(^{a,b})</td>
<td>79.65 (13.73)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>THC</td>
<td>98.40 (9.04)</td>
<td>92.17 (11.22)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>63.44 (7.20)(^{a,b})</td>
<td>58.60 (10.77)(^{a,b})</td>
</tr>
<tr>
<td></td>
<td>MDMA/THC</td>
<td>81.40 (11.14)</td>
<td>79.10 (8.92)</td>
</tr>
</tbody>
</table>

Data represent mean (SEM) expressed as a percentage of vehicle group means. Absolute 5-HT values for vehicle-treated rats were 196.68 (18.32), 274.46 (25.34) and 461.42 (51.68) for the prefrontal cortex, hippocampus and amygdala, respectively. Absolute 5-HIAA values for vehicle-treated rats were 346.22 (40.16), 341.30 (37.82) and 678.58 (74.00) for the prefrontal cortex, hippocampus and amygdala, respectively.

\(^{a}\) \( P<0.05 \) relative to vehicle group.

\(^{b}\) \( P<0.05 \) relative to THC group.

\(^{c}\) \( P<0.05 \) relative to MDMA/THC group, Student Newman–Keuls post-hoc tests.\(^{a}\) \( P<0.05 \) relative to vehicle group.
3.2.3. Neurochemical analysis

HPLC results are presented in Table 5. One-way ANOVA revealed a group effect for 5-HT in the prefrontal cortex ($F(4, 35) = 6.49$, $P < 0.01$), hippocampus ($F(4, 35) = 7.09$, $P < 0.001$) and amygdala ($F(4, 35) = 14.27$, $P < 0.001$). There was also an overall group effect for 5-HIAA in the prefrontal cortex ($F(4, 35) = 7.734$, $P < 0.001$), hippocampus ($F(4, 35) = 6.74$, $P < 0.001$) and amygdala ($F(4, 35) = 12.24$, $P < 0.001$). There were no significant differences between groups in dopamine concentrations in any of the brain areas examined ($Fs < 0.92$).

Post-hoc analyses revealed significantly lower 5-HT and 5-HIAA levels in both the MDMA and MDMA/CP0.1 groups relative to the vehicle group in all brain regions examined. There was also significantly lower 5-HT and 5-HIAA in the hippocampus and amygdala in the MDMA/CP0.2 group relative to the vehicle group. The MDMA/CP0.2/SR group also had significantly lower 5-HT and 5-HIAA levels than the vehicle group in the amygdala.

A partially protective effect of CP 55,940 was suggested by significantly lower 5-HT and 5-HIAA levels in the MDMA group relative to the MDMA/CP0.2 group in the prefrontal cortex. In addition, the MDMA group displayed significantly lower 5-HT levels relative to the MDMA/CP0.2 group in the amygdala. The MDMA group also showed significantly lower 5-HT and 5-HIAA levels relative to the MDMA/CP0.2/SR group in the prefrontal cortex and hippocampus. There were also significantly lower 5-HT levels in the MDMA group relative to the MDMA/CP0.2/SR group in the amygdala.

The MDMA/CP0.1 group displayed significantly lower levels of 5-HIAA compared to both the MDMA/CP0.2 and MDMA/CP0.2/SR groups in the prefrontal cortex.
3.3. Experiment 3: the effect of co-administered SR 141716 on MDMA-induced hyperactivity, hyperthermia and 5-HT depletion

3.3.1. Body temperature

Body temperature changes on the 2 days of drug administration are depicted in Fig. 3. For day 1, ANOVA revealed significant differences between groups for highest \( F(3,28) = 15.77, P < 0.0001 \) and lowest \( F(3,28) = 3.73, P < 0.05 \) body temperature recorded (expressed as change from pre-drug baseline). Results for day 2 revealed significant group differences for highest \( F(3,28) = 9.10, P < 0.001 \) but not lowest \( F(3,28) < 1 \) body temperature recorded. Post-hoc analyses revealed significant hyperthermia in both the MDMA and MDMA/SR groups relative to the vehicle and SR groups. The MDMA/SR group showed a more pronounced hyperthermia, particularly on day 1, when this group differed from the three other groups on maximal hyperthermia.

3.3.2. Locomotor activity

Locomotor activity on the two drug administration days is depicted in Fig. 3. On day 1 and day 2, ANOVA revealed a significant overall group effect \( F(3,28) = 4.55, P < 0.05 \) and \( F(3,28) = 7.60, P < 0.001 \). Post-hoc analyses revealed that there was significantly greater activity in the MDMA group relative to the vehicle and SR groups. The MDMA/SR group showed significantly higher activity than the SR group.

### 3.3.3. Neurochemical analysis

Results from the neurochemical analysis are presented in Table 6. One-way ANOVA revealed an overall group effect for 5-HT in the prefrontal cortex \( F(3,28) = 14.22, P < 0.001 \), hippocampus \( F(3,28) = 15.13, P < 0.001 \) and amygdala \( F(3,28) = 8.73, P < 0.001 \). Significant group differences in 5-HIAA were also seen in the prefrontal cortex \( F(3,28) = 9.98, P < 0.001 \), hippocampus \( F(3,28) = 9.88, P < 0.001 \) and amygdala \( F(3,28) = 4.07, P < 0.05 \). There were no significant differences between groups in dopamine concentrations in any of the brain areas examined \((F < 0.47)\).

Post-hoc analyses revealed significantly lower 5-HT and 5-HIAA in the MDMA and MDMA/SR groups relative to the vehicle and SR group in all brain regions examined.

### 4. Discussion

The present study indicates that co-administration of the main psychoactive constituent of cannabis (THC) or the synthetic cannabinoid CP 55,940 prevents the hyperthermia and partially attenuates the long-term 5-HT depletion produced by MDMA. Furthermore, these results indicate that the CB1 receptor antagonist SR 141716, while reversing cannabinoid agonist effects on MDMA-induced hyperthermia, does not prevent the prophylactic effects of cannabinoid agonists on MDMA-induced 5-HT depletion.

MDMA produced hyperthermia as has been reported in numerous previous studies (Green et al., 2003; Gurtman et al., 2002; Malberg and Seiden, 1998; Morley et al., 2001). On the other hand, administration of THC caused a modest hyperthermia, this effect also being well described in the existing literature (Arnold et al., 2001; Fennessy and Taylor, 1978; Malone and Taylor, 1998, 2001; Pryor et al., 1978). Interestingly, the combination of THC with MDMA induced greater hyperthermia than THC given alone, particularly in the first 2 h of testing. A similar robust hyperthermia was also evident when the effect synthetic cannabinoid agonist CP 55,940 was combined with MDMA. Co-administration of the CB1 antagonist SR 141716 prevented this hyperthermia, suggesting the involvement of CB1 receptors in the effect.

The hyperthermic effect of combined MDMA and cannabinoids resembles that previously seen with d-amphetamine, where 1–4 mg/kg of the stimulant potentiated the hyperthermic effects of 2.5 and 5 mg/kg of THC (Pryor et al., 1978). THC-induced hyperthermia is also potentiated by co-administration of the 5-HT1A receptor antagonist WAY 100635 or the 5-HT reuptake inhibitor fluoxetine and it has been suggested that treatments that increase 5-HT release in the
The hypothalamus may increase THC-induced hypothermia (Malone and Taylor, 1998, 2001). The present results support this hypothesis, with MDMA presumably acting to increase synaptic 5-HT levels in key thermoregulatory centres within the preoptic and anterior hypothalamic regions.

MDMA caused pronounced locomotor stimulation while co-administration of THC or CP 55,940 significantly reversed this hyperactivity. This effect was more pronounced on the second day of testing, presumably due to the novelty of the testing environment on day 1 overcoming some of the inhibitory locomotor effects of cannabinoids. This suppressant effect of cannabinoids on MDMA stimulated activity is consistent with studies showing the ability of CP 55,940 to reduce cocaine and opioid-induced hyperactivity (Arnold et al., 1998; Norwood et al., 2003). Furthermore, pre-treatment with the cannabinoid antagonist SR 141716 attenuated this cannabinoid-induced reversal of hyperactivity, consistent with findings that SR 141716 pre-treatment blocks THC hypoactivity (Compton et al., 1996). The long-term consequences of brief exposure to MDMA (Bull et al., 2003; Fone et al., 2002; Gurtman et al., 2002; McGregor et al., 2003a,b; Morley et al., 2001). In the emergence test, rats pre-

Table 6
Regional changes in 5-HT and 5-HIAA concentrations in Experiment 3 (n = 8 per group)

<table>
<thead>
<tr>
<th>Region</th>
<th>Treatment</th>
<th>5-HT (μg/g)</th>
<th>5-HIAA (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>SR</td>
<td>105.60 (7.00)</td>
<td>111.40 (11.25)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>60.61 (8.71)a,b</td>
<td>67.95 (10.46)a,b</td>
</tr>
<tr>
<td></td>
<td>MDMA/SR</td>
<td>53.85 (6.01)a,b</td>
<td>55.79 (4.95)a,b</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>SR</td>
<td>95.18 (6.23)</td>
<td>95.26 (6.27)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>55.69 (6.94)a,b</td>
<td>55.80 (7.29)a,b</td>
</tr>
<tr>
<td></td>
<td>MDMA/SR</td>
<td>51.90 (6.35)a,b</td>
<td>50.43 (11.37)a,b</td>
</tr>
<tr>
<td>Amygdala</td>
<td>SR</td>
<td>98.79 (10.57)</td>
<td>95.06 (15.44)</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>63.66 (6.08)a,b</td>
<td>61.75 (7.45)a,b</td>
</tr>
<tr>
<td></td>
<td>MDMA/SR</td>
<td>54.97 (6.71)a,b</td>
<td>54.68 (11.05)a,b</td>
</tr>
</tbody>
</table>

Data represent the mean (SEM) expressed as a percentage of the vehicle group means. Absolute 5-HT values of vehicle-treated rats were 364.65 (18.41), 419.77 (25.83) and 745.18 (27.89) for the prefrontal cortex, hippocampus and amygdala, respectively. Absolute 5-HIAA values of vehicle-treated rats were 266.55 (15.60), 414.86 (27.69) and 479.25 (31.19) for the prefrontal cortex, hippocampus and amygdala, respectively.

P < 0.05 relative to vehicle group.

P < 0.05 relative to MDMA group, Student Newman–Keuls post-hoc tests.
MDMA in the emergence test but not in the social interaction test. In contrast, there were no significant differences in the MDMA/THC group relative to controls in the emergence test. Thus, co-administered THC offered some protection against the long-term anxiogenic effects of MDMA in this test.

Rats pre-treated with MDMA also showed decreased social interaction with a novel conspecific relative to both THC- and vehicle-treated animals. Interestingly, the MDMA/THC group also displayed this effect. These results clearly differ from those found on the emergence test, although it is clear from previous studies that certain treatments can have divergent effects in these two tests. For example, acute administration of MDMA increases social interaction in rats, but causes profound inhibition of open field exploration in the emergence test (Morley and McGregor, 2000). Further, chronic administration of fluoxetine appears to reverse the lasting anxiogenic effects of MDMA in this test.

Interestingly, decreased social interaction has been reported in MDMA pre-treated rats in the absence of apparent 5-HT depletion (Fone et al., 2002; McGregor et al., 2003a) suggesting that 5-HT depletion may be sufficient but not necessary to produce long-term changes in social interaction. This finding is in partial agreement with the present results where the MDMA/THC group showed a significant decrease in social interaction without the same comprehensive pattern of 5-HT depletion observed in the MDMA group.

MDMA caused a substantial depletion of 5-HT and 5-HIAA in various brain regions consistent with previous findings from our laboratory and elsewhere (Green et al., 2003; Gurtman et al., 2002; McGregor et al., 2003a,b; O’Shea et al., 1998). Particularly striking here was the observation that rats given either THC or the higher dose of CP 55,940 in conjunction with MDMA displayed 5-HT and 5-HIAA levels in most brain regions that were intermediate between MDMA treated and vehicle treated rats. This suggests some degree of protection afforded by CP 55,940 and THC against MDMA-induced neurotoxicity. The lower co-administered dose of CP 55,940 (0.1 mg/kg) was largely ineffective in preventing MDMA-induced 5-HT depletion, suggesting that the protective effect of cannabinoids require relatively large co-administered doses.

The mechanism underlying the attenuation of MDMA-induced 5-HT depletion by THC and CP 55,940 is of obvious interest. The ability of cannabinoids to prevent MDMA-induced hyperthermia would, at first glance, appear important given earlier findings that preventing MDMA-induced hyperthermia attenuates neurotoxicity (Colado et al., 2001; Malberg et al., 1996; Malberg and Seiden, 1998; O’Shea et al., 2001). However, recent evidence from our laboratory suggests that older Wistar rats are resistant to the protective effects of lower ambient temperatures on MDMA-induced neurotoxicity (McGregor et al., 2003b). Moreover, in the current study, pre-treatment with SR 141716 blocked the CP 55,940 reversal of MDMA-induced hyperthermia but did not change the partial protection against 5-HT depletion. This indicates that prevention of hyperthermia may not be the critical factor in cannabinoid-mediated protective effects.

The most likely mechanism underlying the protective effects of cannabinoids may therefore, involve their antioxidant properties. MDMA-induced neurotoxicity requires free radical formation since antioxidant drugs that block this process are neuroprotective (Colado and Green, 1995; Shankaran et al., 2001). Evidence suggests that phenolic cannabinoids such as THC and CP 55,940 have a structural resemblance to the powerful antioxidant vitamin E (Chen and Buck, 2000; Hampson et al., 1998). Cannabinoids have antioxidant effects in vitro and are neuroprotective in animal models of stroke (Leker et al., 1999; Nagayama et al., 1999; Shohami and Mechoulam, 2000). In many studies, a CB1-independent mechanism has been highlighted, through sometimes an ineffectiveness of SR 141716 pre-treatment or through a protective effect being evident in CB1 knockout mice (Chen and Buck, 2000; Marsicano et al., 2002; Martinez-Orgado et al., 2003; Nagayama et al., 1999). The mechanism of neuroprotection in the latter instances has been suggested to be due to antioxidant properties that are mediated by the phenolic moiety of these cannabinoids.

In conclusion, the current results indicate that co-administered cannabinoids may attenuate the 5-HT depletion and some of the anxiety-related behaviours that are observed weeks after MDMA administration in rats. Our observations indicate a CB1-independent mechanism of neuroprotection possibly through countering oxidative stress, although further studies are required to directly examine these issues. Our results do not however, suggest that human MDMA users should resort to THC consumption as a harm minimization strategy. Firstly, the protective doses of THC and CP 55,940 used in the current study were high and it is unlikely that these effects would be obtained with the relatively small amounts of THC typically consumed during recreational cannabis use. Secondly, the effect of cannabinoid administration on MDMA-induced neurotoxicity in cannabinoid-tolerant animals is unknown: thus protection from the neurotoxic effects of MDMA may not necessarily be obtained in frequent cannabis users. Finally, it should be stressed that the neuroprotective effects of THC and CP 55,940 were by
no means complete, with only partially protective effects evident in all brain regions examined.

Acknowledgements

This work was supported by a National Health and Medical Research Council grant to ISM and GEH and an Australian Research Council grant to ISM and PEM. Kirsten Morley is the recipient of an Australian Postgraduate Award.

References


Shankaran, M., Yamamoto, B.K., Gudelsky, G.A., 2001. Ascorbic acid prevents 3,4-methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT. Synapse 40, 55–64.


