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Haloperidol, but not clozapine, produces dramatic catalepsy in Δ^9-THC-treated rats: possible clinical implications

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1 The effect on rat catalepsy induced by Δ^9-tetrahydrocannabinol (Δ^9-THC) in association with haloperidol (HP) or clozapine (CLOZ) administration was investigated.

2 Δ^9-THC dose-dependently increased HP (0.05–1 mg kg^{-1}, s.c.)-induced rat catalepsy, while no catalepsy was observed after CLOZ (1–20 mg kg^{-1}, s.c.) or Δ^9-THC + CLOZ administration.

3 The CB_1 antagonist SR141716A (0.5–5 mg kg^{-1}, i.p.) reversed the increase mediated by Δ^9-THC on HP-induced catalepsy.

4 The D_2 agonist quinpirole completely reversed the catalepsy induced by both HP and HP + Δ^9-THC; however, higher doses of quinpirole were needed in the presence of Δ^9-THC.

5 The M_1 antagonist scopolamine and α_2 antagonist yohimbine were able to reduce the catalepsy induced by HP and HP + Δ^9-THC in a similar manner.

6 CLOZ and the 5-$HT_{2A/2C}$ antagonists ritanserin, RS102221 and SB242084 were more effective in antagonizing HP than HP + Δ^9-THC-induced catalepsy.

7 HP and CLOZ failed to inhibit *in vitro* [^{3}H]CP-55,940 binding, while Δ^9-THC and SR141716A did not show an appreciable affinity for the D_2 receptor.

8 It was suggested that the different effects on rat catalepsy induced by Δ^9-THC following HP or CLOZ administration may depend on the receptor-binding profiles of the two antipsychotics.

9 The preferential use of CLOZ rather than HP in the treatment of psychotic symptoms in cannabis abusers was discussed.

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Abbreviations: CLOZ, clozapine; Δ^9-THC, Δ^9-tetrahydrocannabinol; EPS, extrapyramidal side effects; HP, haloperidol

Introduction

Considerable research has indicated that cannabis use may precipitate psychosis-like symptoms among vulnerable individuals. Moreover, it has been shown that cannabis use increases the risk of relapse, as well as worsens and prolongs the psychotic symptoms among patients who have already developed the disorder (for review, see Degenhardt & Hall, 2002).

Antipsychotic drugs are largely employed in the management of schizophrenia and other psychotic disorders; however, a possible interaction between cannabis and antipsychotic administration has not been adequately investigated. Depending on their pharmacological properties, the antipsychotics currently used in clinical practice are divided into conventional antipsychotics, such as haloperidol (HP), and atypical antipsychotics, such as clozapine (CLOZ). The clinical improvements observed in patients treated with conventional antipsychotics are often accompanied by the appearance of extrapyramidal side effects (EPS), such as akinesia, rigidity and tremors. The blockade of the dopamine D_2 receptor in the basal ganglia has been associated with the development of EPS (Kapur & Remington, 2001). Atypical antipsychotics are able, in different grades, to antagonize the striatal dopamine D_2 receptor at therapeutic doses; however, they have shown low or no propensity to induce EPS (Tarsi *et al*., 2002). Depending on which author is being read, this peculiarity of atypical antipsychotics was associated with a favorable D_2/M_1 (Haraguchi *et al*., 1997), D_2/5-$HT_{2A/2C}$ (Meltzer *et al*., 1989; Reavill *et al*., 1999), or D_2/α_2 receptor antagonism ratio (Kalkman *et al*., 1998). It was, indeed, hypothesized that a receptor-binding profile similar to that observed for CLOZ might counteract the dopamine D_2 receptor blockade effect in the basal ganglia, leading to a reduced incidence of EPS.

Like D_2 receptor blockade, cannabinoid CB_1 receptor stimulation is known to affect motor activity. Consistently, several researches involving volunteers given CB_1 agonists in the laboratory showed impairment in a variety of motor tasks (Yesavage *et al*., 1985; Wilson *et al*., 1994). In rats, dose-dependent ataxia and catalepsy were observed after the systemic administration of cannabinoids (Chaperon & Thiebot, 1999). Furthermore, cannabinoid agonists microinjected into the striatum produced rat immobility (Gough & Olley, 1978). Within the central nervous system, the D_2 and CB_1 receptors are densely expressed in the basal ganglia (Herkenham *et al*., 1991), and colocalization between the two receptors

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has been indicated in the rat striatum (Hermann *et al*., 2002). In a previous study, Anderson *et al*. (1996) showed that the cannabinoid agonist CP-55,940 exacerbated the catalepsy induced by the D_2 receptor antagonist raclopride. The aim of the present study was to determine the effect of HP or CLOZ on rat catalepsy following the administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the most psychoactive component of marijuana.

Methods

Animals

Male Sprague–Dawley rats weighing 200–250 g (Charles River, Calco, Italy) were housed at 22 ± 2°C on a 12 h light/dark cycle (light on at 0700, off at 1900), with food available *ad libitum*. All experimental protocols were accepted by the Ethical Committee of Cagliari University in accordance with the EC regulation for the care and use of experimental animals (EEC No. 86/609).

Rat catalepsy

Catalepsy, defined as the acceptance and retention of abnormal posture, was measured by means of the *bar test*. Briefly, rat forepaws were gently placed over a horizontal bar, fixed at a height of 10 cm above the working surface. The length of time during which the animal ($n=8$–10 for each group) retained this position was recorded by an observer blind to the treatment, and by measuring the time elapsing from the placement of the rat until the removal of one of its forepaws. Rats were removed from the bar if their latency on the *bar test* exceeded 300 s (i.e. cutoff = 300 s). Results were expressed as percentage vs the cutoff time (i.e. catalepsy % = latency on *bar test* (s)/cutoff (300 s) × 100).

Considering that stress and novelty may affect the results by reducing the rat latency on *bar test*, the longest latency time of three consecutive trials was recorded and considered more reliable in determining the rat acceptance of abnormal posture. Rats were injected with the different drugs in their home cage, where they were maintained until the three consecutive trials on *bar test* latency were scored (90 min after antipsychotic injection).

Homogenate binding

[^{3}H]CP-55,940 (180 Ci mmol^{-1}; NEN, Boston, MA, U.S.A.) binding was performed by a modification of the method previously described (Rinaldi-Carmona *et al*., 1994). Briefly, a P2 fraction of rat cerebral (minus cerebellum) membranes (50–80 μg of protein) was incubated with 0.5 nM of [^{3}H]CP-55,940 for 1 h at 30°C in a final volume of 0.5 ml of TME buffer (50 mM Tris-HCl, 1 mM EDTA and 3.0 mM $MgCl_2$, pH 7.4) containing 5 mg ml^{-1} of BSA. Nonspecific binding was estimated in the presence of 1 μM of CP-55,940 or WIN-55,212-2 (1 μM) (Tocris Cookson Ltd). All binding studies were performed in disposable glass tubes pretreated with Sigma-Cote (Sigma Chemical Co. Ltd, Poole, U.K.). The reaction was terminated by rapid filtration through Whatman GF/C filters presoaked in 0.5% polyethyleneimine, using a Brandell 96-sample harvester (Gaithersburg, MD, U.S.A.). Filters were washed five times with 4 ml aliquots of ice-cold Tris-HCl buffer (pH 7.4) containing 1 mg ml^{-1} BSA.

[^{3}H]YM-09151-2 (85 Ci mmol^{-1}; NEN) binding was determined by the method developed by Niznik *et al*. (1985). Briefly, 200 μl of extensively washed striatal membrane (50–75 μg protein) was added to the incubation medium (50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM EDTA and 5.7 mM ascorbic acid, pH 7.4), containing 50 pM of [^{3}H]YM-09151-2. Nonspecific binding was determined by adding (−)-Sulpiride (10 μM) (Sigma Chemical Co.). After 60 min incubation at 25°C in the dark, the samples were filtered through Whatman GF/B filters. Filters were rinsed four times with 4 ml of ice-cold Tris-HCl buffer, pH 7.4.

Radioactivity was measured in a liquid scintillation counter (Tricarb 2900, Packard, Meridien, U.S.A.) using 3 ml of scintillation fluid (Ultima Gold MV, Packard). Protein determination was performed by means of a protein assay (Bio-Rad, Milan, Italy) using BSA as a standard, according to the supplier's protocol. All competition-binding assays were performed in triplicate using 8–10 different concentrations of the drug to be tested. Results were confirmed in at least four independent experiments. Data were analyzed using the Kell 6.0 program (Biosoft, Ferguson, MO, U.S.A.).

Statistical analyses

The statistical significance of the effect of the different treatments was evaluated by two-way analysis of variance (ANOVA). When a significant interaction ($P<0.05$) was demonstrated, the Newman–Keuls *post hoc* test was applied in order to compare the effects induced by the different administrations.

Drugs and chemicals

HP-HCl, CLOZ, RS102221, yohimbine and quinpirole were purchased from Tocris Cookson Ltd (Avonmouth Bristol, U.K.). Ritanserin and Δ^9-THC were from Sigma Co. (St Louis, MO, U.S.A.). SR141716A and SB242084 were generously provided by Sanofi-Synthélabo (Bagneaux, France) and by SmithKline Beecham (Harlow, U.K.), respectively. Δ^9-THC, dissolved in a 1 : 1 : 18 emulphor/ethanol/saline solution, was administered i.p. in a volume of 1 ml kg^{-1}. The remaining drugs were dissolved in 25 μl of glacial acetic acid, buffered (pH 6.5) using Na_2CO_3 (0.1 M) in distilled water and, depending on the drug (see below), administered s.c. in a volume of 1 ml kg^{-1} or i.p. in a volume of 5 ml kg^{-1}.

Δ^9-THC was always administered (i.p.) 15 min before antipsychotic injection (s.c.). Quinpirole was administered (i.p.) 30 min after antipsychotic injection, while, considering the pharmacokinetic parameters, the remaining drugs were administered (i.p.) 20 min before Δ^9-THC injection. When Δ^9-THC, clozapine and HP were coadministered, HP was injected (s.c.) 30 min after clozapine (s.c.) and 15 min after Δ^9-THC administration (i.p.).

Results

Rat catalepsy

Effect of Δ^9-THC in association with HP or CLOZ As shown in Figure 1 (top panel), Δ^9-THC (0.5 mg kg^{-1}, i.p.)

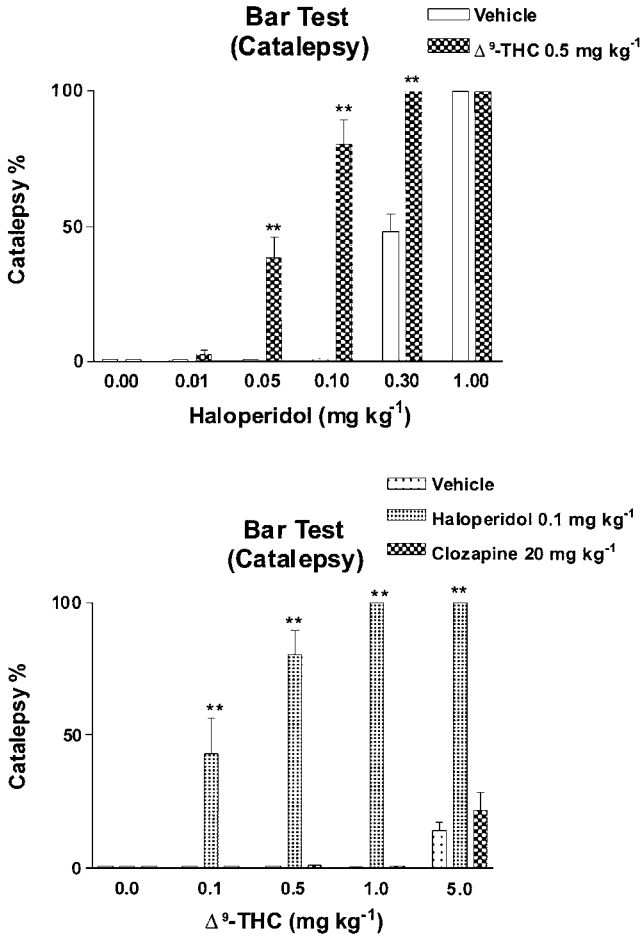

Figure 1 (Top panel) Effect of Δ^9-THC (0.5 mg kg^{-1}, i.p.) on rat *bar test* latency in association with different doses of HP. (Bottom panel) Histogram showing the effect on *bar test* latency of different doses of Δ^9-THC in association with HP (0.1 mg kg^{-1}, s.c.) or CLOZ (20 mg kg^{-1}, s.c.). Rats ($n=8$) were injected (i.p.) with Δ^9-THC 15 min prior HP (s.c.) or CLOZ (s.c.) administration. Rat catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA (top panel, $F_{HP\ dose}(5, 84)=210.9$, $P<0.01$; $F_{\Delta 9\text{-}THC}(1, 84)=158.1$, $P<0.01$; $F_{interact}(5, 84)=35.9$, $P<0.01$) (bottom panel, $F_{\Delta 9\text{-}THC\ dose}(4, 103)=42.14$, $P<0.01$; $F_{HP}(2, 103)=286.6$, $P<0.01$; $F_{interact}(8, 103)=24.69$, $P<0.01$), followed by Newman–Keuls *post hoc* test (top panel; $^{**}P<0.01$ vs rat receiving the same HP dose + vehicle) (bottom panel; $^{**}P<0.01$ vs rat treated with the same Δ^9-THC dose + vehicle).

increased the latency on the *bar test* in rats treated with different doses of HP (0.01–1 mg kg^{-1}, s.c.) (HP + vehicle $ED_{50}=0.29$ mg kg^{-1}, confidence 0.27–0.33; HP + Δ^9-THC $ED_{50}=0.049$ mg kg^{-1}, confidence 0.017–0.08), while no differences compared to vehicle-treated rats were observed when Δ^9-THC was associated with CLOZ (0.5–20 mg kg^{-1}, s.c.) (data not shown). Δ^9-THC dose-dependently increased the latency on the *bar test* in rats treated with a dose of HP (0.1 mg kg^{-1}, s.c.), which *per se* did not induce significant catalepsy, while no differences were observed when the same doses of Δ^9-THC were associated with CLOZ (20 mg kg^{-1}, s.c.) (Figure 1, bottom panel). During both pretest (home cage) and test section, rats treated with Δ^9-THC + HP showed strong rigidity associated with an increased vocalization and a reduced fecal bolus production.

Effect of SR141716A on Δ^9-THC-mediated potentiation of HP-induced catalepsy In order to determine whether Δ^9-THC affected HP-induced catalepsy through the activation of the CB_1 receptor, the cannabinoid antagonist SR141716A was administered before (20 min) Δ^9-THC administration. SR141716A (0.5–5 mg kg^{-1}, i.p.) reversed the potentiation of HP-induced catalepsy produced by Δ^9-THC (0.5 mg kg^{-1}, i.p.) (Figure 2). High doses of SR141716A (3–5 mg kg^{-1}, i.p.) were unable to completely reverse HP + Δ^9-THC-induced catalepsy, and no significant differences between HP- and HP + Δ^9-THC + SR141716A- (3–5 mg kg^{-1}, i.p.) treated rats were observed ($P>0.05$). Consistently, SR-141716A administration failed to affect the catalepsy induced by HP (Figure 2).

Effect of quinpirole on Δ^9-THC-mediated potentiation of HP-induced catalepsy The D_2-receptor agonist quinpirole dose-dependently reversed both HP- and HP + Δ^9-THC-induced catalepsy. However, the disappearance of rat catalepsy occurred at a higher dose of quinpirole in HP + Δ^9-THC-treated rats compared to HP-treated rats (1.5 vs 1 mg kg^{-1}, respectively) (Figure 3).

Effect of M_1, α_2 and/or 5-$HT_{2A/2C}$ receptor antagonists on Δ^9-THC-mediated potentiation of HP-induced catalepsy Considering that CLOZ may have a low propensity to induce rat catalepsy because of its antagonistic activity on the M_1, α_2 and/or 5-$HT_{2A/2C}$ receptor, the effect of the blockade of such receptors on HP- or HP + Δ^9-THC-induced catalepsy was also investigated.

The administration of different doses of scopolamine (M_1) (Figure 4, top panel) or yohimbine (α_1) (Figure 4, bottom panel) reduced rat catalepsy induced by HP or HP + Δ^9-THC in a similar manner, since no significant differences ($P>0.05$) were observed when comparing the effect induced by such drugs in HP- vs HP + Δ^9-THC-treated rats (Figure 4). Conversely, the three mixed 5-$HT_{2A/2C}$ antagonists ritanserin, (Figure 5, top panel), RS10221 and SB242084 (Figure 5,

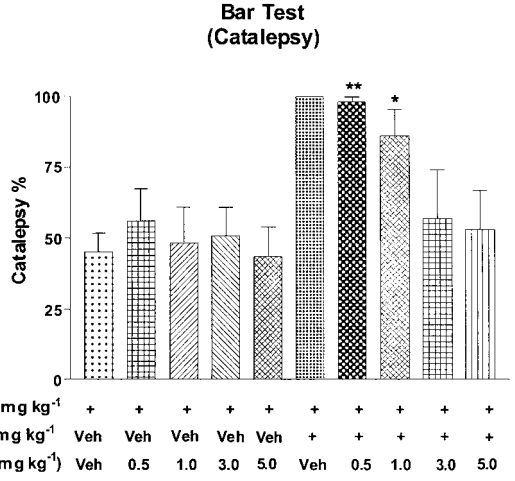

Figure 2 Histogram showing the effect of increasing doses of SR141716A on *bar test* latency induced by HP (0.3 mg kg^{-1}, s.c.) ± Δ^9-THC (0.5 mg kg^{-1}, i.p.). SR141716A (i.p.) injection was carried out 20 min before Δ^9-THC administration and 35 min before HP. Rat catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA ($F_{SR\text{-}141716A\ dose}(4, 99)=4.89$, $P<0.05$; $F_{\Delta 9\text{-}THC}(1, 81)=17.5$, $P<0.01$; $F_{interact}(4, 81)=4.72$, $P<0.05$), followed by the Newman–Keuls *post hoc* test ($^{*}P<0.05$; $^{**}P<0.01$ vs HP + vehicle + vehicle-treated rats).

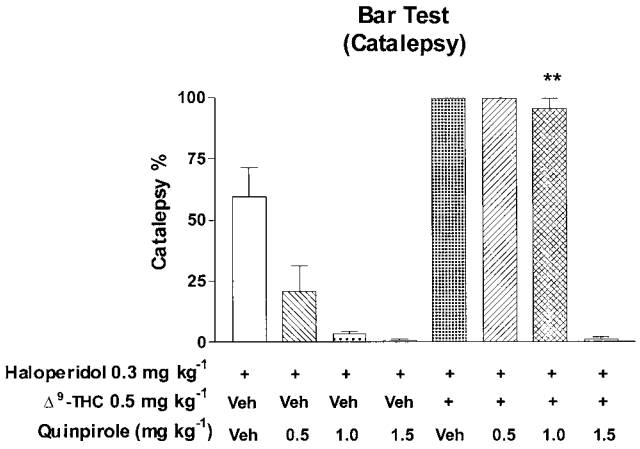

Figure 3 Histogram showing the effect of quinpirole on the *bar test* latency induced by HP (0.3 mg kg^{-1}, s.c.) or HP + Δ^9-THC (0.5 mg kg^{-1}, i.p.). Rats ($n = 10$) were injected (i.p.) with quinpirole 30 min after HP injection (s.c.) and 45 min after Δ^9-THC administration (i.p.). Rat catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA ($F_{\text{quinpirole dose}}(3, 72) = 22.17$, $P < 0.01$; $F_{\Delta 9\text{-THC}}(1, 72) = 40.93$, $P < 0.01$; $F_{\text{interact}}(3, 72) = 7.06$, $P < 0.01$), followed by the Newman–Keuls *post hoc* test (**$P < 0.01$ vs HP + quinpirole (1 mg kg^{-1}) + vehicle-treated rats).

bottom panel) showed a reduced ability to alleviate HP-induced catalepsy after the coadministration of Δ^9-THC.

Effect of clozapine on Δ^9-THC-mediated potentiation of HP-induced catalepsy CLOZ was able to reverse both HP- and HP + Δ^9-THC-induced catalepsy, showing a U-shaped curve (Figure 6). However, higher doses of CLOZ were needed to completely reverse the catalepsy induced by HP + Δ^9-THC, than were needed to reduce catalepsy induced by HP (5 vs 1 mg kg^{-1} of CLOZ, respectively) (Figure 6).

Homogenate binding

[^{3}H]YM-09151-2 and [^{3}H]CP-55,940 bindings were clearly saturable, showing a K_d of 23.5 ± 0.3 pM in the [^{3}H]YM-09151-2-binding assay and a K_d of 0.32 ± 0.05 nM in the [^{3}H]CP-55,940-binding assay. Similar results were obtained when WIN55,212-2 (1 μM) was substituted to CP-55,940 in defining [^{3}H]CP-55,940 nonspecific binding, indicating that [^{3}H]CP-55,940 selectively bound the CB_1 receptor.

The results of the [^{3}H]YM-09151-2 and [^{3}H]CP-55,940 competition-binding assays are shown in Table 1. HP and CLOZ failed to inhibit the *in vitro* [^{3}H]CP-55,940 binding, while Δ^9-THC and SR141716A did not show an appreciable affinity for the D_2 receptor.

Discussion

The present results showed a different effect on rat catalepsy induced by Δ^9-THC when coadministered with HP or with CLOZ. The cataleptogenic properties of HP are related to the degree of D_2-receptor blockade induced by the antipsychotic in the basal ganglia (Kapur & Remington, 2001). The increased catalepsy exerted by the association of HP and Δ^9-THC is thus in agreement with the potentiation of rat catalepsy induced by the D_2-antagonist raclopride following CP-55,940 administration (Anderson *et al*., 1996). Furthermore, Δ^9-THC was shown to potentiate the motor impairment induced by the depletion of striatal dopamine following reserpine administration (Moss *et al*., 1981). Our results indicated that the effect induced by Δ^9-THC on HP-induced catalepsy was mediated by the stimulation of CB_1 receptors, since the cannabinoid antagonist SR141716A was able to reverse the Δ^9-THC effect. SR141716A was not able to affect HP-induced rat catalepsy, as also observed in raclopride-induced catalepsy (Anderson *et al*., 1996), indicating that the catalepsy induced by the D_2 blockade does not involve the stimulation of the CB_1 receptors in its mechanism of action. Differently from SR141716A, the administration of the D_2 agonist quinpirole completely reversed both HP- and HP + Δ^9-THC-induced catalepsy, even

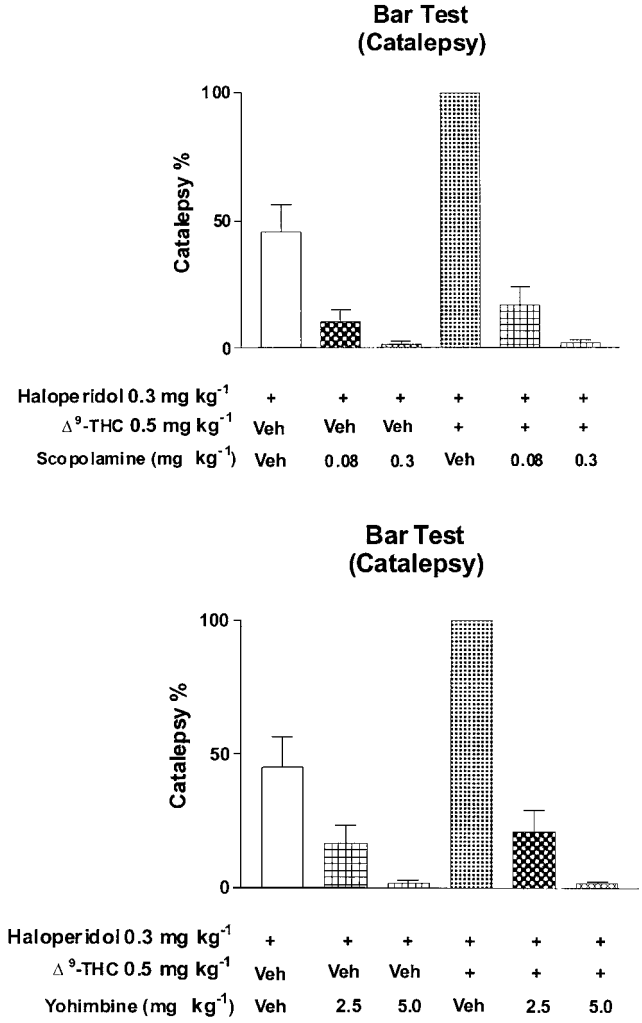

Figure 4 Effect of scopolamine (top panel) or yohimbine (bottom panel) on rat *bar test* latency induced by HP (0.3 mg kg^{-1}) or HP + Δ^9-THC (0.5 mg kg^{-1}). Scopolamine or yohimbine injections (i.p.) were carried out 20 min before Δ^9-THC administration (i.p.) and 35 min before HP (s.c). Rat ($n = 10$) catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA (top panel, $F_{\text{scopolamine dose}}(2, 54) = 49.49$, $P < 0.01$; $F_{\Delta 9\text{-THC}}(1, 54) = 9.91$, $P < 0.01$; $F_{\text{interact}}(2, 54) = 7.57$, $P < 0.01$) (bottom panel, $F_{\text{yohimbine dose}}(2, 54) = 38.56$, $P < 0.01$; $F_{\Delta 9\text{-THC}}(1, 54) = 8.18$, $P < 0.01$; $F_{\text{interact}}(2, 54) = 6.78$, $P < 0.01$), followed by the Newman–Keuls *post hoc* test (top panel; *$P < 0.05$; **$P < 0.01$ vs rat treated with the same scopolamine dose + HP + vehicle) (bottom panel; *$P < 0.05$; **$P < 0.01$ vs rat treated with the same yohimbine dose + HP + vehicle).

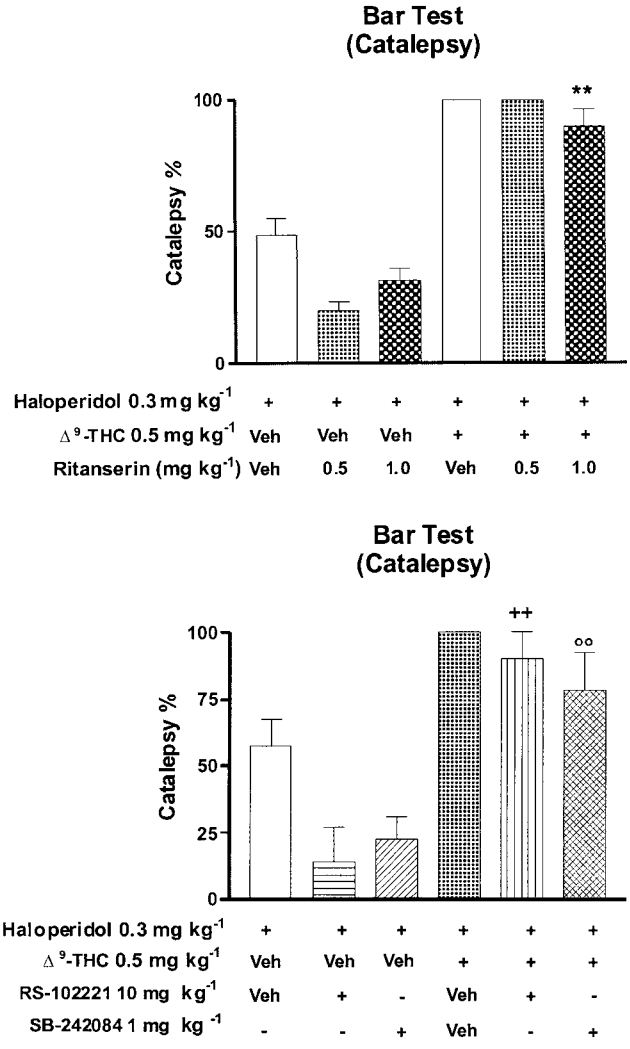

Figure 5 Effect of ritanserin (top panel), RS-10221 or SB-242084 (bottom panel) on rat *bar test* latency induced by HP (0.3 mg kg^{-1}) or HP + Δ^9-THC (0.5 mg kg^{-1}). 5-HT$_{2A/2C}$ antagonist injections (i.p.) were carried out 20 min before Δ^9-THC administration (i.p.) and 35 min before HP (s.c.). Rat ($n = 10$) catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA (top panel; $F_{ritanserin\ dose}(1, 56) = 5.36$, $P < 0.05$; $F_{\Delta 9\text{-}THC}(1, 56) = 192.1$, $P < 0.01$; $F_{interact}(2, 56) = 4.69$, $P < 0.05$) (bottom panel; $F_{drug}(1, 56) = 5.16$, $P < 0.05$; $F_{\Delta 9\text{-}THC}(1, 56) = 20.46$, $P < 0.01$; $F_{interact}(2, 56) = 4.51$, $P < 0.05$), followed by the Newman–Keuls *post hoc* test (**$P < 0.01$ vs HP + ritanserin (1 mg kg^{-1}) + vehicle-treated rats; $^{++}P < 0.01$ vs HP + RS102221 + vehicle-treated rats; $^{\circ\circ}P < 0.01$ HP + SB242084 + vehicle-treated rats).

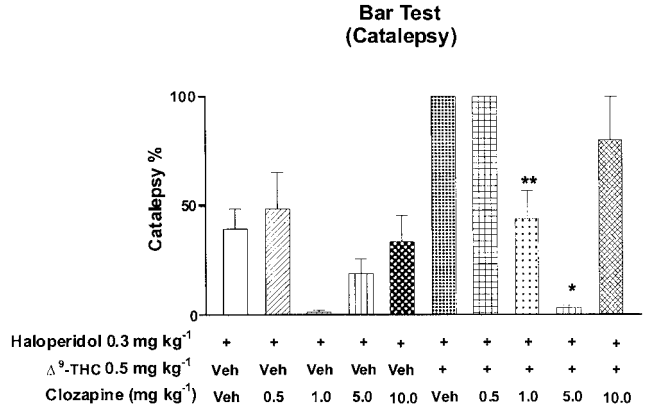

Figure 6 Histogram showing the effect of CLOZ on the *bar test* latency induced by HP (0.3 mg kg^{-1}, s.c.) or HP + Δ^9-THC (0.5 mg kg^{-1}, i.p.). Rats ($n = 10$) were injected (s.c.) with CLOZ 30 min before HP injection (s.c.) and 15 min prior to Δ^9-THC administration (i.p.). Rat catalepsy was scored 90 min after antipsychotic administration. Data were analyzed using two-way ANOVA ($F_{clozapine\ dose}(4, 99) = 11.90$, $P < 0.01$; $F_{\Delta 9\text{-}THC}(1, 99) = 9.46$, $P < 0.01$; $F_{interact}(4, 99) = 4.69$, $P < 0.05$), followed by the Newman–Keuls *post hoc* test (**$P < 0.01$ vs HP + CLOZ (1 mg kg^{-1}) + vehicle-treated rats; *$P < 0.05$ vs HP + CLOZ (5 mg kg^{-1}) + vehicle-treated rats).

Table 1 K_i of different drugs on [^{3}H] YM-09151 (D_2) and on [^{3}H]CP55,940 (CB1) binding

Drug	D_2 K_i (nM) ± s.e.m.	CB_1 K_i (nM) ± s.e.m.
Haloperidol	1.11 ± 0.04	> 10,000
Clozapine	151 ± 4.5	> 10,000
Δ^9-THC	> 10,000	21.2 ± 0.81

Data represent mean ± s.e.m. of least four independent experiments. Competition-binding assays were carried out using 8–10 different concentrations for each K_i determination. [^{3}H]YM-09151-2 and [^{3}H]CP-55,940 binding assays were used in drug-interaction studies on D_2 and CB_1 receptors, respectively.

if higher doses of quinpirole were needed in the presence of Δ^9-THC. Our binding studies *at equilibrium* did not support the possibility that Δ^9-THC may act through a direct blockade of the dopamine D_2 receptor; however, the possibility that the CB_1 and D_2 receptors may interact at the signal transduction level is under consideration (Glass & Felder, 1997; Meshler & Howlett, 2001). Different studies have indicated that CB_1 agonists reduced the stimulatory effect mediated by dopamine on motor behavior (Moss *et al*., 1981). For instance, it has been shown that the cannabinoid receptor agonist WIN55,212-2 reduced the quinpirole alleviation of akinesia in reserpine-treated rats (Manuef *et al*., 1997). Furthermore, the anandamide transport inhibitor *N*-(4-hydroxyphenyl)-arachidonamide (AM404) reduced the stimulation of motor behaviors elicited by the D_2 receptor agonist quinpirole in rats (Beltramo *et al*., 2000).

HP exerted a cataleptic effect only when high levels of striatal D_2 receptors were blocked (Kapur & Remington, 2001), indicating that even a low stimulation produced by the endogenous dopamine is able to counteract the motor disturbance induced by the antipsychotic. Here we showed that doses of HP that normally did not induce catalepsy became cataleptogenic in the presence of Δ^9-THC. It is feasible that the reduced efficacy of the D_2 agonism in the basal ganglia following CB_1 receptor stimulation may account for a stronger effect produced by HP on rat catalepsy.

In spite of the discrete D_2-receptor occupancy observed after CLOZ treatment (Kapur & Remington, 2001), the coadministration of Δ^9-THC and CLOZ did not exert catalepsy. CLOZ is known to have low or no propensity to induce EPS in both humans and laboratory animals; furthermore, it has been shown that CLOZ possesses the ability to reduce rat catalepsy induced by D_2 antagonists (Young *et al*., 1999; Isacson *et al*., 2002). As mentioned above, such anticataleptic properties of CLOZ have been related to the blockade of the muscarinic M_1, serotonergic $5HT_{2A/2C}$ and adrenergic α_2 receptors. The muscarinic antagonist scopolamine and the α_2 antagonist

yohimbine reversed both HP- and HP + Δ^9-THC-induced catalepsy in a similar manner, indicating that the blockade of such receptors was still efficient in lowering catalepsy after Δ^9-THC administration. Accordingly, CLOZ reversed both HP- and HP + Δ^9-THC-induced catalepsy, suggesting that the association of CLOZ and Δ^9-THC did not produce rat catalepsy because some of the anticataleptic properties of the antipsychotic were maintained.

The use of three different mixed $5HT_{2A/2C}$ antagonists indicated that the ability of the 5-$HT_{2A/2C}$ receptor blockade lost potency in reducing rat catalepsy when HP was associated with Δ^9-THC administration. The exact nature of such different interactions can only be speculated on the present results. One possible explanation might depend on the altered release of serotonin after CB_1 agonist administration, which has been observed in several areas (Nakazi *et al*., 2000; Egashira *et al*., 2002). Furthermore, it has been shown that the 5-$HT_{2A/2C}$ antagonist ritanserin reduced HP-induced catalepsy only when moderate cataleptogenic doses of the antipsychotic were used (Reavill *et al*., 1999), suggesting that when high levels of catalepsy were reached, the blockade of 5-$HT_{2A/2C}$ receptor loses its ability to modulate the motor disturbance. Possibly, the high level of catalepsy induced by the association of HP and THC may in itself account for the reduced ability of the 5-$HT_{2A/2C}$ to lessen rat catalepsy.

The reduced efficacy of the 5-$HT_{2A/2C}$ blockade relied on the observed lower ability of CLOZ to reverse HP-induced catalepsy following Δ^9-THC administration; however, further studies are needed to determine whether other receptor systems may be involved in such a phenomenon.

To date, the effect of antipsychotic medication in cannabis-addicted patients has been inadequately investigated. A reduced efficacy of HP was observed by Knudsen & Vilmar (1984) in schizophrenic patients assuming cannabis, suggesting a possible cannabis–HP antagonistic interaction in the treatment of psychotic symptoms. Furthermore, a comparative study between HP and olanzapine in the treatment of cannabis-induced psychotic disorders indicated that, while the atypical antipsychotic olanzapine was as effective as HP, a higher rate of EPS was associated with HP treatment (Berk *et al*., 1999). Although some authors have indicated that not all the interactions between dopamine and the cannabinoid system observed in rodents may be extended to primates, the present results, obtained from an animal model of human EPS, support the preferential use of CLOZ rather than HP in the treatment of mental disorders in cannabis abusers. Furthermore, the possibility that the administration of cannabinoid agonists with antipsychotics might be a useful tool in discriminating between typical and atypical antipsychotics cannot be excluded.

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