Cannabinoid Receptor Antagonists Counteract Sensorimotor Gating Deficits in the Phencyclidine Model of Psychosis

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Clinical and laboratory findings suggest that cannabinoids and their receptors are implicated in schizophrenia. The role of cannabinoids in schizophrenia remains however poorly understood, as data are often contradictory. The primary aim of this study was to investigate whether the cannabinoid CB1 receptor antagonists rimonabant and AM251 are able to reverse deficits of sensorimotor gating induced by phencyclidine and to mimic the 'atypical' antipsychotic profile of clozapine. The prepulse inhibition (PPI) of the startle reflex was used to measure deficits of sensorimotor gating. PPI-disruptive effects of phencyclidine and their antagonism by rimonabant, AM251, and clozapine were studied in rats. The effects of rimonabant were carefully examined taking into account dose ranges, vehicle, and route of administration. We also examined the ability of rimonabant to reduce the PPI-disruptive effects of dizocilpine and apomorphine. Rimonabant as well as AM251 significantly counteracted the phencyclidine-disruptive model of PPI, comparable to the restoring effect of clozapine; no augmentation effect was observed with rimonabant and clozapine as cotreatment. Rimonabant also significantly attenuated the PPI disruptive effects of dizocilpine and apomorphine. Taken together, our results indicate that CB1 receptor antagonists do produce 'atypical' antipsychotic profile mimicking that of clozapine in the phencyclidine disruption of sensorimotor gating. Our findings further suggest that CB1 receptor antagonism may be involved in restoring disturbed interactions between the activity of the endocannabinoid system and glutamate neurotransmitter system implied in schizophrenia.

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INTRODUCTION

Cannabis sativa is one of the most frequently abused substances among schizophrenic patients (Jablensky *et al*, 1992). Although some researchers have interpreted this phenomenon to hypothesize cannabis consumption as a method of self-therapy for counterbalancing negative symptoms of schizophrenia or side effects of antipsychotic treatment (Dixon *et al*, 1991; Krystal *et al*, 1999), recent meta-analyses of epidemiologic studies have concluded that cannabis use is a risk factor or contributory cause of developing psychosis (Hall, 2006; Henquet *et al*, 2005; Semple *et al*, 2005). Indeed, the impact of cannabis in schizophrenia may greatly vary across subjects, plausibly depending on factors such as individual susceptibility to cannabinoids (Hall, 1998), as well as specific neurobiological differences in cannabinoid circuitry among different diagnostic clusters (Ujike *et al*, 2002). Nevertheless, several lines of evidence suggest the involvement of cannabinoid CB_1 receptors and their endogenous ligands, anandamide and 2-arachidonylglycerol (2-AG), in the pathophysiology of schizophrenia.

CB_1 receptors are mainly distributed in areas of the human brain implicated in schizophrenia, including prefrontal cortex, anterior cingulate cortex, basal ganglia, and hippocampus (Rinaldi-Carmona *et al*, 1996); of note, post-mortem studies have shown increased CB_1 receptor densities in the prefrontal and anterior cingulate cortex of schizophrenics as compared with the normal controls (Dean *et al*, 2001; Ujike and Morita, 2004; Zavitsanou *et al*, 2004). Furthermore, animal studies indicate that cannabinoid CB_1 receptors interact with the main neurotransmitter systems implied in the pathophysiology of schizophrenia, such as dopamine and glutamate (Heinz *et al*, 2003; van der Stelt and Di Marzo, 2003).

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Emerging evidence seems to point to a possible anti-psychotic-like effect of anandamide, as the selective inhibition of its degradation reduces the effect of D2 receptor activation on several behavioral parameters (Beltramo *et al*, 2000; Tzavara *et al*, 2006). Moreover, anandamide levels in cortico-spinal fluid of antipsychotic-naïve schizophrenic patients are markedly elevated and inversely correlated with psychopathological symptoms (Giuffrida *et al*, 2004).

Conversely, 2-AG might contribute to trigger or exacerbate psychotic symptoms by inhibiting glutamate release and signaling in several brain areas through activation of presynaptic cannabinoid CB_1 receptors (Domenici *et al*, 2006; Heresco-Levy, 2005; Piomelli, 2003).

In light of this multifaceted background, it is of great interest to investigate the effects of CB_1 antagonists, in order to assess the role of this receptor in the regulation of the neurobiological mechanisms underlying psychotic phenomena.

One important endophenotype of schizophrenia is the impairment of sensorimotor gating (Gotesman and Gold, 2003). This preattentional function has been defined as a process by which an organism filters extraneous information from external and internal milieu, and its dysfunction is a common feature across the schizophrenia spectrum (Braff *et al*, 2001). The most reliable paradigm for measuring abnormalities of sensorimotor gating is the prepulse inhibition (PPI) of the acoustic startle reflex. Deficits of PPI are seen across the entire spectrum of psychotic disorders (Braff *et al*, 1978, 2001; McGhie and Chapman, 1961). Notably, PPI can be assessed across species using near-identical stimulus parameters (Swerdlow and Geyer, 1998), and changes in this paradigm have been used to predict antipsychotic efficacy (Swerdlow *et al*, 2002). All antipsychotics without distinction between 'typical' and 'atypical' profiles reverse PPI in rats treated with dopamine agonists, such as apomorphine (Martinez *et al*, 2002; Swerdlow and Geyer, 1993, 1998). On the contrary, PPI disruption induced by *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine, is preferentially reversed by clozapine and other 'atypical' antipsychotic agents (Bakshi and Geyer, 1995; Bakshi *et al*, 1994; Geyer and Ellenbroek, 2003; Keith *et al*, 1991; Swerdlow *et al*, 1996).

The primary goal of this study was to examine whether CB1 receptor blockade by two well-validated CB_1 receptor antagonists, such as rimonabant and the even more selective AM251 (Hajos and Freund, 2002; Mato *et al*, 2002) can reverse the gating deficits in the PPI-disruptive model of phencyclidine and parallel the restoring effects of clozapine in this model.

To date, a number of observations concerning the effects of rimonabant on psychosis-relevant behaviors in animals have yielded contrasting results. In particular, in one study rimonabant did not reverse the PPI-disruptive effects of apomorphine, amphetamine, or dizocilpine (Martin *et al*, 2003). However, the phencyclidine animal model, which produces the most robust PPI deficits (Geyer and Ellenbroek, 2003) and has excellent face validity for core psychotic symptoms (Moghaddam and Jackson, 2003), was not used in these studies. In the present investigation, particular attention was given to vehicle used, route of administration and dose ranges of rimonabant in the phencyclidine paradigm. In addition, we re-examined the effects of rimonabant on PPI following disruptions by dizocilpine and apomorphine.

MATERIALS AND METHODS

Animals

A total of 355 male Sprague–Dawley rats (Harlan, Italy) weighing 250–350 g served as subjects in the present study. Rats were housed in groups of two in plastic cages under reversed 12-h light–dark cycle (lights on at 2000 and off at 0800). Methods for housing and all behavioral testing were performed according to the substantial literature of startle measures in rodents (Swerdlow *et al*, 2002). Food and water were available *ad libitum*, and each rat was handled individually within 3 days of arrival and daily thereafter. Throughout all experiments efforts were made to minimize animal suffering. Behavioral testing occurred between 0900 and 1500 (Swerdlow *et al*, 1996), unless otherwise indicated. All experimental procedures were approved by the local institutional Ethical Committee and carried out in strict accordance with the European Community guidelines for care and use of experimental animals (CEE NE86/609).

Drugs and Chemicals

The following drugs were used: phencyclidine hydrochloride (1.25 mg/kg, s.c.); dizocilpine (0.1 mg/kg, s.c.); apomorphine (0.25 mg/kg s.c.); rimonabant (0.75, 1.5, or 3.0 mg/kg, s.c.); AM251 (1.4 or 1.8 mg/kg, s.c.); clozapine (7.5 mg/kg i.p.). Phencyclidine, dizocilpine, apomorphine, and clozapine were purchased from Sigma Aldrich, Italy. Rimonabant was obtained from the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program. AM251 was purchased from Tocris, UK. Phencyclidine and dizocilpine were dissolved in 0.9% saline. Apomorphine was dissolved in saline with 0.1% ascorbic acid. Rimonabant and AM251 were dissolved in ethanol, Tween 80, and 0.9% saline (ratio 1:1:18). Ethanol final concentration was 3.75%; this amount of ethanol does not produce any effect on PPI, as confirmed by the results of a preliminary study performed to compare saline with rimonabant or AM251 (data not shown). All drugs were administered in an injection volume of 1 ml/kg. The doses of phencyclidine, dizocilpine, and apomorphine were chosen because they have been found previously to significantly disrupt PPI in rats (Bakshi *et al*, 1994; Geyer *et al*, 2001; Mansbach and Geyer, 1989; Zhang *et al*, 1999); the doses of rimonabant and AM251 were chosen on the grounds of a preliminary set of dose–response experiments assessing their ability to reverse PPI disruption. All substances were administered at a convenient time interval before experimental testing, compatible with their pharmacokinetic properties, as shown in previous papers and in preliminary experiments, so as to elicit their effects during behavioral testing (Bakshi and Geyer, 1995; De Vries *et al*, 2001; Geyer *et al*, 2001; Swerdlow *et al*, 1996, 2002). All experiments were conducted in separate groups of drug-naive and experimentally naive rats. Each rat was only tested for PPI once, in one drug condition.

Behavioral Testing

Startle experiments used two identical startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA) housed in a sound-attenuated room with a 60-dB ambient noise level. Each chamber consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5×25.5 cm Plexiglas frame within a ventilated enclosure. The delivery of acoustic stimuli was controlled by the SR-LAB microcomputer and interface assembly, which also digitized, rectified, and recorded stabilimeter readings, with 100, 1-ms readings collected beginning at stimulus onset. Startle magnitude was defined as the average of the 100 readings. Acoustic stimuli and background noise were presented via a Radio Shack Supertweeter mounted 24 cm above the Plexiglas cylinder. Startle magnitude was detected and recorded as transduced cylinder movement via a piezoelectric device mounted below the Plexiglas stand. All sound levels within each chamber were calibrated regularly and found to remain constant over the test period (SR-LAB Startle Calibration System).

Testing Procedures

Three days before drug testing each rat was placed into the startle chamber with 70 dB background noise and 5 min later was exposed to 18 pulse-alone (a 40 ms, 120 dB noise burst) trials and six prepulse + pulse (a 20 ms, 82–12 dB over background-burst followed 100 ms later by the 120 dB burst) trials. The purpose of this initial session was to create equally matched treatment groups based on the mean startle magnitude data (from the pulse-alone trials) for each rat (Ballmaier et al, 2001, 2002; Swerdlow et al, 1996).

On testing days, approximately 1 h after arrival in the laboratory, rats were placed in a startle chamber (closed Plexiglas cylinder). Each test session was approximately 20 min long and consisted of 5 min of 70 dB background noise followed by several presentations in a pseudorandom order of five trial types: (1) pulse alone noise burst (a 40 ms 120 dB noise burst); (2) prepulse trials which consisted of 20-ms noise bursts 3, 6, or 12 dB above 70 dB background noise followed 100 ms later by a 120 dB pulse; (3) no stimulus in which only the background noise was presented. The no stimulus trials were used to assess gross motor activity during the test session but were not included in the statistical analysis. There was a total number of 50 trials (12 pulse-alone trials, 10 each of the 3-, 6-, or 12-dB prepulse + pulse trials, eight no stimulus trials). In addition, five pulse alone trials, which were not included in the calculation of PPI values, were presented at the beginning and at the end of the test session to ensure a relatively stable level of startle reactivity for the remainder of the session. Prepulse intensities were chosen to span a range of relatively weak (3 dB) and intense (12 dB) stimuli. Intertrial intervals averaged 15 s.

For experiments assessing the effects with rimonabant, saline or one of the doses of rimonabant (0.75, 1.5, 3.0 mg/kg s.c.) were administered as pretreatment. Rats were then treated as follows: saline or phencyclidine 20 min after pretreatment followed by other 10 min before being placed in the startle chamber for the test session; saline or dizocilpine 15 min after pretreatment followed by other

15 min before being tested; saline or apomorphine 30 min after pretreatment and immediate transfer of the animal in the startle chamber for the test session. For experiments investigating the effects of AM251, rats were pretreated with saline or one of the doses of AM251 (1.4 or 1.8 mg/kg s.c.), followed by saline, or phencyclidine, as stated above. In a series of experiments, clozapine was administered as a pretreatment 30 min before PPI testing. In the clozapine plus rimonabant experiment, rats were pretreated with vehicle or clozapine followed 10 min later by vehicle or rimonabant; after 20 min the rats received phencyclidine and 10 min later each rat was placed in the startle chamber for a test session.

Data Analysis

PPI data were analyzed with the general linear model univariate analysis of variance (ANOVA) using PPI values as dependent variable and pharmacological treatment and prepulse magnitude as fixed factors (statistical package, SPSS version 10). The prepulse magnitude was analyzed at all 3 dB levels. The amount of PPI was defined as the percent reduction in startle magnitude in the presence of the prepulse compared to the magnitude in the absence of the prepulse $(100-(100 \times$ magnitude on prepulse trial/magnitude on pulse trial). Startle magnitude was calculated as the average response to all of the pulse-alone trials. The initial and final five pulse-alone trials were not included in the analysis, in order to ensure the calculation of PPI over a more stable range of startle responses. Startle amplitude data were analyzed with one-way ANOVA. The Bonferroni test was used as a post hoc test for multiple comparisons with $p < 0.05$ as threshold for significant difference.

RESULTS

Effects of Pretreatment with Rimonabant on Phencyclidine-Induced Disruption of PPI and Startle Magnitude

In the first series of experiments, we wanted to test whether the CB antagonist rimonabant affects the phencyclidine-induced deficit of PPI. As a preliminary experiment, we assessed possible effects on PPI of several doses (0.75 mg/kg, $n = 8$, 1.5 mg/kg, $n = 6$, 3.0 mg/kg, $n = 8$, s.c.; saline $n = 5$) of rimonabant and found that this drug does not significantly alter either startle amplitude or PPI when administered alone at the doses tested (Figure 1a and b).

Then, we tested three doses of rimonabant (0.75, 1.5, or 3.0 mg/kg s.c., administered 30 min before PPI procedure) on phencyclidine (1.25 mg/kg, s.c., administered 10 min before PPI procedure) induced disruption of PPI (Figure 2a). Rats treated with phencyclidine showed a significant decrease in PPI with respect to saline-treated rats (all groups $n = 5$/group), (two-way ANOVA, Groups: $F(4, 60) = 15.06$ $p < 0.001$; Prepulse intensity: $F(2, 60) = 22.19$ $p < 0.001$; Groups × Intensity: $F(8, 60) = 0.26$ NS; post hoc Bonferroni's test: phencyclidine vs sal $p < 0.001$) (Figure 2a). Pretreatment with rimonabant at all doses tested significantly counteracted phencyclidine-induced decrease in PPI (rimonabant0.75 + phencyclidine vs phencyclidine $p < 0.001$, rimonabant1.5 + phencyclidine vs
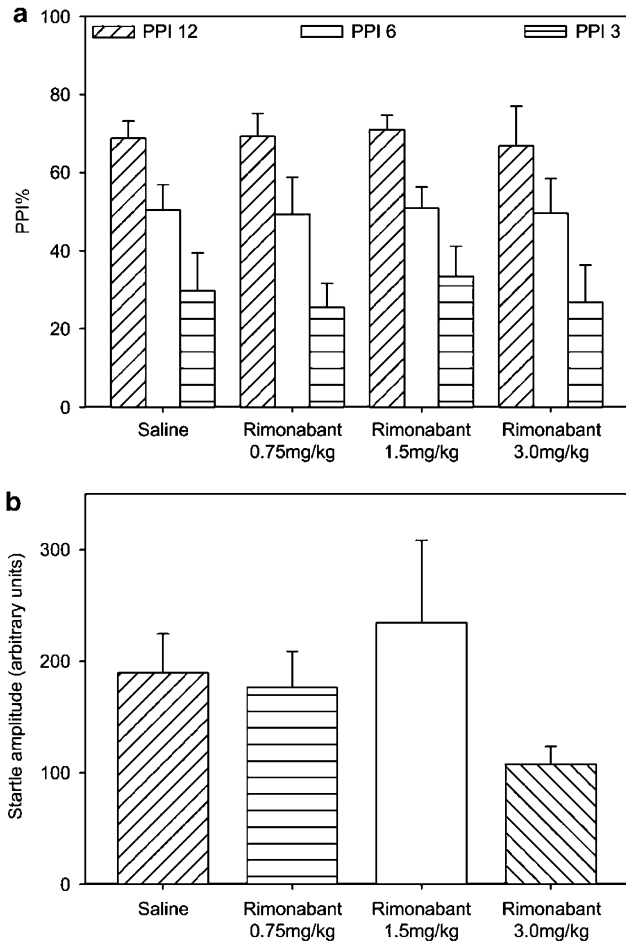
Figure 1 (a) Effects of rimonabant (0.75–3.0 mg/kg, s.c.) on PPI. (b) Effects of rimonabant (0.75–3.0 mg/kg, s.c.) on startle amplitude.

Figure 2 (a) Effects of rimonabant (0.75–3.0 mg/kg, s.c.) on disruption of PPI induced by phencyclidine (1.25 mg/kg, s.c.). (b) Effects of rimonabant (0.75–3.0 mg/kg, s.c.) and/or phencyclidine (1.25 mg/kg, s.c.) on startle amplitude. PCP = phencyclidine.

phencyclidine $p < 0.001$, rimonabant3.0 + phencyclidine vs phencyclidine $p = 0.018$) (Figure 2a). No drug treatment significantly altered startle amplitude (Figure 2b). This finding was re-examined carrying out the behavioral testing between 1500 and 2100 with different groups of animals ($n = 5$/group). The results (not shown) revealed the same level of significance as described above.

In order to confirm that the effects of rimonabant on phencyclidine-induced disruption of PPI are due to its activity on CB1 receptors, we repeated the experiment using AM251, a more selective CB1 receptor antagonist, at doses equivalent to the median effective dose, as determined in our studies of rimonabant. AM251 (1.4, 1.8 mg/kg s.c., administered 30 min before PPI procedure) significantly counteracted phencyclidine-induced disruption of PPI (all groups $n = 5$/group; two-way ANOVA, Groups: $F(3, 48) = 21.35$ $p < 0.001$; Prepulse intensity: $F(2, 48) = 65.60$ $p < 0.001$; Groups × Intensity: $F(6, 48) = 1.77$ NS; post hoc Bonferroni's test: phencyclidine vs sal $p < 0.001$, AM251 1.4 + phencyclidine vs phencyclidine $p = 0.003$, AM251 1.8 + phencyclidine vs phencyclidine $p < 0.001$, AM251 1.4 + phencyclidine vs sal $p < 0.001$, AM251 1.8 + phencyclidine vs sal $p = 0.079$) (Figure 3a). No drug treatment significantly affected startle amplitude (Figure 3b).
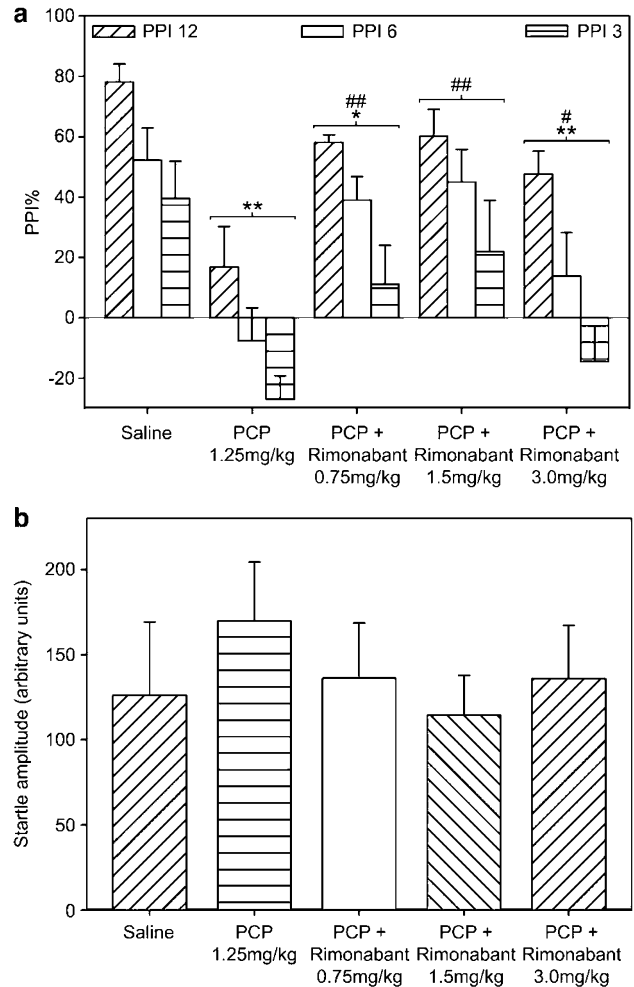
The effects of rimonabant on phencyclidine-induced disruption of PPI were compared with those of the atypical antipsychotic clozapine. Rimonabant (1.5 mg/kg s.c., administered 30 min before PPI procedure) and clozapine (7.5 mg/kg i.p., administered 30 min before PPI procedure) treatments as well as rimonabant + clozapine cotreatment significantly counteracted phencyclidine effects on PPI (all groups $n = 12$/group; two-way ANOVA, Groups: $F(4, 165) = 27.64$ $p < 0.001$; Prepulse intensity: $F(2, 165) = 87.82$ $p < 0.001$; Groups × Intensity: $F(8, 165) = 1.78$ NS; post hoc Bonferroni's test: phencyclidine vs sal $p < 0.001$, rimonabant + phencyclidine vs phencyclidine $p < 0.001$, clozapine + phencyclidine vs phencyclidine $p < 0.001$, rimonabant + clozapine + phencyclidine vs phencyclidine $p < 0.001$) and were significantly different from saline treatment (rimonabant + phencyclidine vs sal $p < 0.001$, clozapine + phencyclidine vs sal $p = 0.034$, rimonabant + clozapine + phencyclidine vs sal $p < 0.001$) (Figure 4a). However, there was no additive effect of rimonabant + clozapine pretreatment with respect to rimonabant or clozapine pretreatments alone (rimonabant + phencyclidine + clozapine vs clozapine + phencyclidine or rimonabant + phencyclidine NS)
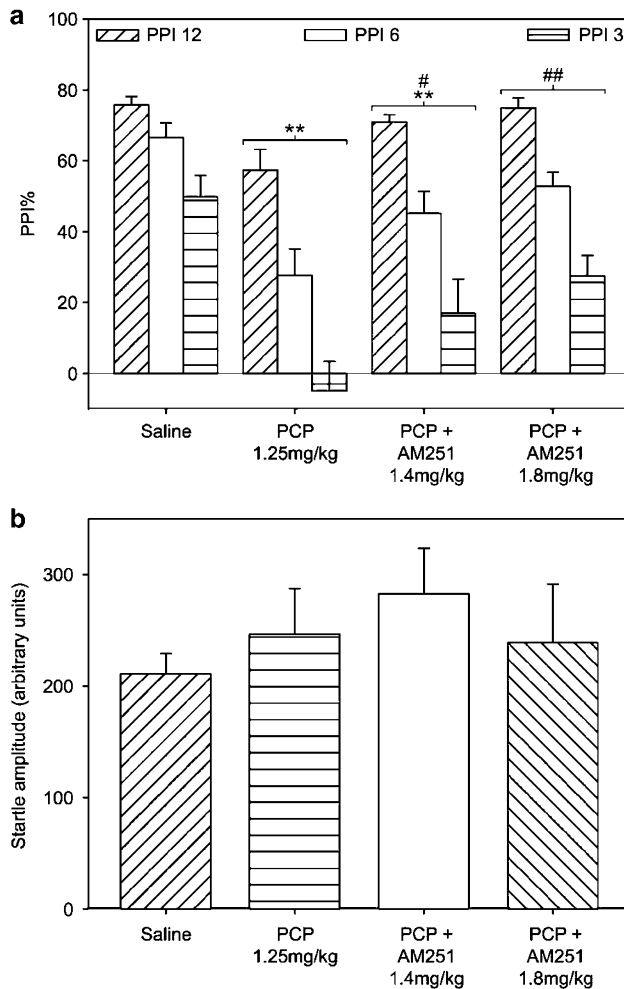
Figure 3 (a) Effects of AM251 (1.4, 1.8 mg/kg, s.c.) on disruption of PPI induced by phencyclidine (1.25 mg/kg, s.c.), (b) Effects of AM251 (1.4, 1.8 mg/kg, s.c.) and/or phencyclidine (1.25 mg/kg, s.c.) on startle amplitude. PCP = phencyclidine.
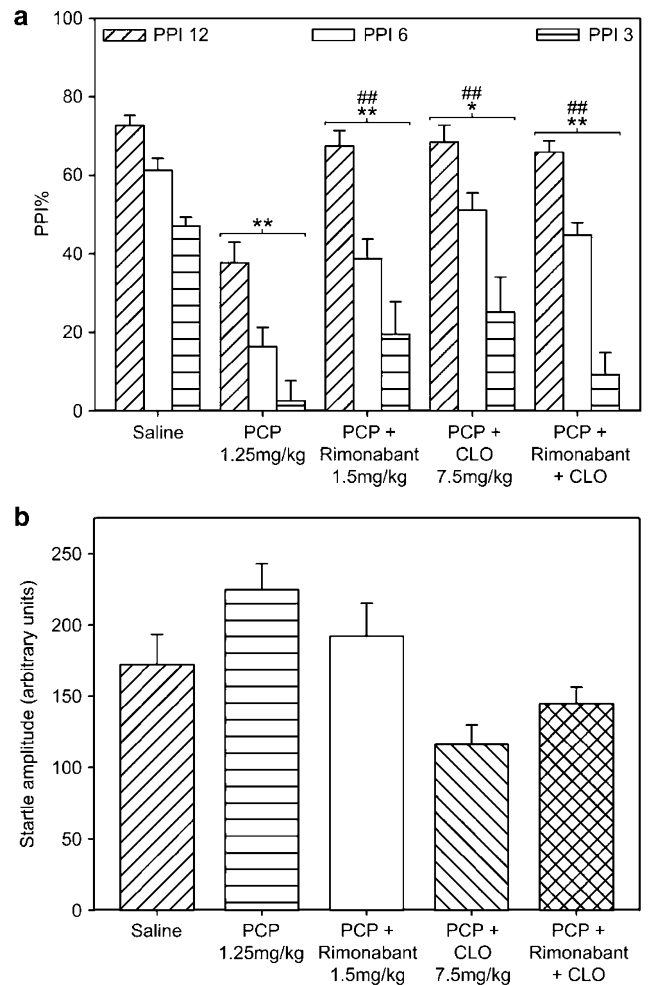
Figure 4 (a) Effects of rimonabant (1.5 mg/kg, s.c.) or clozapine (7.5 mg/kg, i.p.) on disruption of PPI induced by phencyclidine (1.25 mg/kg, s.c.). (b) Effects of rimonabant (1.5 mg/kg, s.c.) and/or phencyclidine (1.25 mg/kg, s.c.) and/or clozapine (7.5 mg/kg, i.p.) on startle amplitude. CLO = clozapine; PCP = phencyclidine.

(Figure 4a). No drug treatment altered startle amplitude with respect to saline treatment (Figure 4b).

Effects of Pretreatment with Rimonabant on Dizocilpine-Induced Disruption of PPI and Startle Magnitude

In order to substantiate the results observed on phencyclidine in the PPI paradigm, we studied the effects of rimonabant pretreatment (1.5 mg/kg, s.c.) on PPI disruption induced by dizocilpine (0.1 mg/kg s.c., administered 15 min before PPI procedure), another NMDA noncompetitive antagonist. As already shown, dizocilpine significantly decreased PPI with respect to saline treatment (all groups $n = 16$/group; two-way ANOVA, Groups: $F_{(3, 180)} = 110.08$ $p < 0.001$; Prepulse intensity: $F_{(2, 180)} = 46.52$ $p < 0.001$; Groups × Intensity: $F_{(6, 180)} = 2.01$ NS; *post hoc* Bonferroni's test: dizocilpine *vs* sal $p < 0.001$) (Figure 5). Rimonabant did not alter PPI but significantly counteracted dizocilpine disruption of PPI (rimonabant *vs* sal NS, rimonabant + dizocilpine *vs* dizocilpine $p < 0.001$, rimonabant + dizocilpine *vs* sal $p < 0.001$) (Figure 5). No drug treatment altered

startle amplitude with respect to saline treatment (not shown).

Effects of Pretreatment with Rimonabant on Apomorphine-Induced Disruption of PPI and Startle Amplitude

In a second series of experiments, we tested whether rimonabant (1.5 mg/kg, administered 30 min before PPI procedure) prevents PPI disruption induced by apomorphine (0.25 mg/kg s.c., administered immediately before the PPI procedure), a nonselective dopaminergic agonist. Apomorphine significantly decreased PPI with respect to saline treatment (all groups $n = 15$/group; two-way ANOVA, Groups: $F_{(3, 168)} = 84.74$ $p < 0.001$; Prepulse intensity: $F_{(2, 168)} = 6.76$ $p = 0.001$; Groups × Intensity: $F_{(6, 168)} = 1.99$ NS; *post hoc* Bonferroni's test: apomorphine *vs* sal $p < 0.001$), whereas rimonabant did not alter PPI (Figure 6a). As already shown for its effects on phencyclidine- or dizocilpine-disruption of PPI (see above), rimonabant significantly counteracted apomorphine-induced decrease of PPI (rimonabant + apomorphine *vs* apomorphine
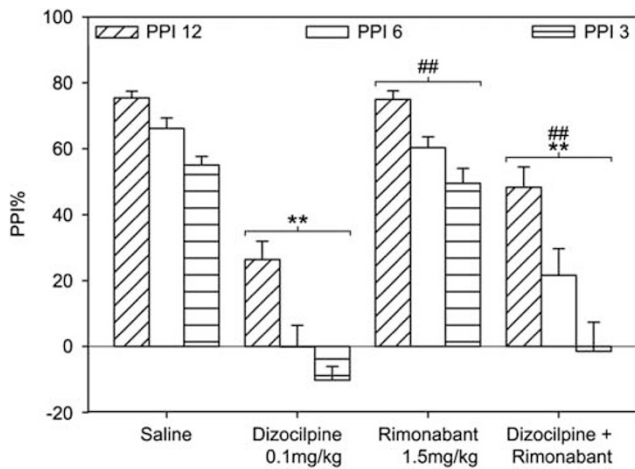
Figure 5 Effects of rimonabant (1.5 mg/kg, s.c.) on PPI disruption induced by dizocilpine (0.1 mg/kg, s.c.).

Figure 6 (a) Effects of rimonabant (1.5 mg/kg, s.c.) on PPI disruption induced by apomorphine (0.25 mg/kg, s.c.). (b) Effects of rimonabant (1.5 mg/kg, s.c.) and/or apomorphine (0.25 mg/kg, s.c.) on startle amplitude. APO = apomorphine.

$p < 0.001$, rimonabant + apomorphine *vs* sal $p < 0.001$) (Figure 6a). No drug treatment significantly altered startle amplitude with respect to saline treatment (Figure 6b).

DISCUSSION

The main result of the present study is that the potent CB_1 receptor antagonist rimonabant significantly counteracted PPI deficits induced by phencyclidine at all doses tested. Notably, the apparent lack of dose dependency for such an effect may likely reflect a dose range that omits lower, less effective doses of rimonabant in our experiments. To rule out the possibility that rimonabant might mediate these effects by engaging an as yet unidentified non-CB_1 cannabinoid receptor (Breivogel *et al*, 2001), we also tested the highly specific CB_1 antagonist AM251, obtaining similar results. Of note, both CB_1 antagonists paralleled the ability of clozapine to antagonize the disruption of PPI induced by phencyclidine. Finally, rimonabant markedly reduced PPI deficits induced by dizocilpine, another NMDA antagonist, and apomorphine. No significant effects on startle magnitude were produced by any experiment. In sum, the results indicate that rimonabant exerts behavioral effects in pharmacological models of disrupted sensorimotor gating consistent with 'atypical' antipsychotic profile, and that these effects are mediated through blockade of the CB_1 receptor. These findings are in agreement with previous evidence for antipsychotic-like properties of rimonabant, such as the ability to suppress the hyperactivity induced in gerbils by various psychotomimetic agents (Poncelet *et al*, 1999) and to attenuate memory impairments produced by Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis, in rats (Mallet and Beninger, 1998).

Both rimonabant and AM251 are known to act as CB_1 inverse agonists at relatively high doses (see, eg, Haller *et al*, 2004), raising the possibility that some of the observed results may not simply depend on CB_1 blockade. Such a hypothesis seems to be ruled out by the significant effects elicited by lower doses of rimonabant (0.75–1.5 mg/kg, i.p.), at which this compound has been shown to exert no
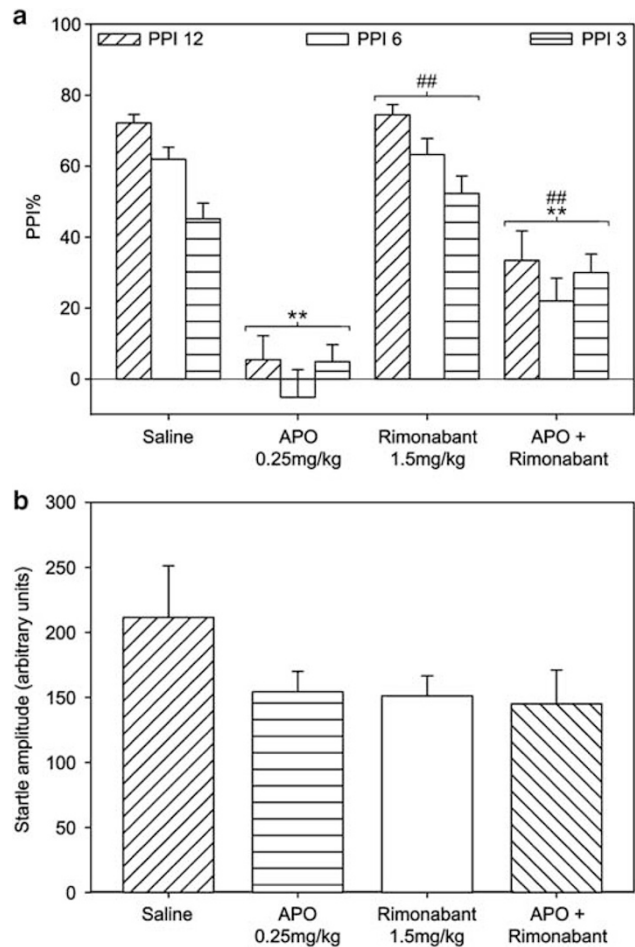
intrinsic activity on CB_1 receptors in rodent behavior (see, eg, Bortolato *et al*, 2006). Of note, such a possibility is also suggested by the lack of PPI effects induced by rimonabant *per se* in our study.

Part of our finding is in apparent contrast with a previous study by Martin *et al* (2003), who reported that rimonabant had no effect on PPI following disruption by dizocilpine, apomorphine and *d*-amphetamine. This discrepancy may be explained by the doses range used, the preparation of rimonabant, and the route of administration. Indeed, in our preliminary experiments we followed the experimental conditions of the study by Martin *et al* (2003) by administering rimonabant as a suspension of 0.5% carboxymethylcellulose via intraperitoneal injections. Under these experimental conditions inconsistent results were observed on the ability of rimonabant to reverse PPI disruption induced by NMDA antagonists and DA agonists (not shown). Although this might reflect a different influence of the route of administration on rat behaviors (Melzacka *et al*, 1979), we also considered the possibility that the vehicle used in that study was inadequate to fully dissolve rimonabant, owing to the highly lipophylic properties of the drug. Thus, we applied the recommendations of the

National Institute of Mental Health Synthesis and Drug Supply Program including a high-impact study by DeVries et al (2001), who dissolved rimonabant in ethanol, Tween 80 and sterile saline (ratio 1 : 1 : 18) and administered the drug s.c., in order to investigate the effects of the CB_1 antagonist on the prevention of relapse to cocaine use. Following the protocol by deVries et al (2001), we were able to produce consistent and reproducible results.

In addition to differences in drug preparation, administration and dose range, differences in the parameters used for measurements of startle gating might also account for the discrepancy with the previous report. Martin et al (2003) used 5-, 11- and 17-dB prepulses over a background intensity of 68 dB, whereas we used a more sensitive threshold range of PPI produced by prepulse intensities of 3-, 6-, and 12 dB above a background of 70 dB. Previous studies have shown that deficits in PPI are most sensitively detected using weak prepulse stimuli rather than intense prepulse stimuli, thereby avoiding floor and ceiling effects (Swerdlow and Geyer, 1993). This observation merits further investigation and is in accordance with our results suggesting that PPI deficits in states of gating circuit dysfunction might be most sensitively detected in conditions in which the system can exhibit the greatest changes.

The NMDA receptor antagonist phencyclidine is frequently used to model schizophrenia in laboratory animals. This compound induces hyperlocomotion in the open field (Gleason and Shannon, 1997), hyperlocomotion and social deficit in the social withdrawal model (Corbett et al, 1995; Sams-Dodd, 1995), alters swimming patterns in the water maze (Ogawa et al, 1994; Okuyama et al, 1995), and increases immobility in the forced swimming test after chronic administration (Noda et al, 1995). Importantly, phencyclidine-induced disruption of PPI is a paradigm of sensorimotor gating abnormalities, which currently appear to be at the core of certain aspects of behavioral pathophysiology in the spectrum of schizophrenia-like disorders.

Although our findings suggest that the PPI-disruptive mechanisms of phencyclidine, dizocilpine, and apomorphine involve the activation of CB_1 receptors, they do not allow to identify the neurobiological mechanisms underlying the antipsychotic-like actions of rimonabant. However, the ability of this drug to attenuate phencyclidine- and dizocilpine-mediated PPI disruptions—both absolutely insensitive to D_2 receptor antagonists—suggests that rimonabant might exert its antipsychotic-like properties through nondopaminergic mechanisms. Several studies have reported that CB_1 agonists can reduce glutamatergic synaptic transmission in several brain regions involved in the regulation of gating functions, such as the hippocampus (Fujiwara and Egashira, 2004; Misner and Sullivan, 1999), the prefrontal cortex (Auclair et al, 2000), the nucleus accumbens (Robbe et al, 2001) and the amygdala (Azad et al, 2003). Interestingly, the bulk of evidence identifies the endocannabinoid 2-AG as the most probable retrograde mediator in long-term plasticity at glutamatergic synapses (Gerdeman et al, 2002; Robbe et al, 2002; Sjöström et al, 2003; Straiker and Mackie, 2005). In particular, 2-AG is synthesized in the postsynaptic terminal of glutamatergic synapses following activation of metabotropic mGLUR5 glutamate receptors (Jung et al, 2005) and is thought to transiently suppress glutamate release through activation of CB_1 receptors located in presynaptic glutamatergic axon terminals (Katona et al, 2006). Notably, mGLUR5 receptors are known to modulate the psychotomimetic actions of NMDA receptor antagonists (Kinney et al, 2005) and their pharmacological or genetic impairment induces psychosis-like behavior in animals (Brody et al, 2004; Kinney et al, 2003). In light of these premises, it is possible to speculate that inhibition of CB_1 presynaptic receptors might limit the effects of 2-AG, thus enhancing glutamate release and neurotransmission on mGLUR5 and other glutamatergic postsynaptic receptors and limiting the PPI-disrupting impact of NMDA antagonists. Such a mechanism would also be in agreement with in vivo microdialysis studies indicating that THC inhibits the release of glutamate in the hippocampus and that this inhibition is antagonized by rimonabant (Fujiwara and Egashira, 2004). Thus, the behavioral effects of rimonabant that we observed could reflect inhibition of phencyclidine-induced decrease of glutamatergic neurotransmission.

A similar mechanism of action might also account for the ability of rimonabant to attenuate the deficit induced by apomorphine downstream the dopaminergic synapse. Changes in glutamatergic release are likely to affect the role of dopaminergic activation in PPI (Bortolato et al, 2005) and the positive modulation of glutamatergic transmission inhibits dopamine-mediated behaviors in the rats (Dall'Olio et al, 1994). Alternatively, rimonabant might reduce the apomorphine-mediated disruption through independent mechanisms, acting beyond the dopaminergic synapse. Further studies are warranted to better understand the role of CB1 receptors in apomorphine-mediated PPI disruption.

Apparently, our data are in contrast with previous literature highlighting an antipsychotic-like role of anandamide, the other CB1 receptor endogenous ligand (Beltramo et al, 2000; Giuffrida et al, 2004). Indeed, selective inhibition of anandamide degradation seems to have a protective role against the behavioral effects of d-amphetamine, including PPI disruption (Beltramo et al, 2000; Bortolato et al, in preparation). Preliminary studies suggest that anandamide may be implicated in the intrinsic regulation of dopamine release, by acting retrogradely on CB_1 receptors located in a subset of dopaminergic presynaptic terminals to limit dopamine release. Interestingly, however, anandamide seems to have no effect on glutamate release. Under this perspective, it is possible to speculate that anandamide and 2-AG might subserve different physiological mechanisms and play different roles in the pathophysiology of schizophrenia, probably in parallel to the different mechanisms underlying psychosis. The intrinsic actions of rimonabant observed in this study might be plausibly consistent with a prevalent blockade of 2-AG actions. This effect is supported by observations attesting concentrations of 2-AG approximately 1000 times higher than anandamide in the brain (nanomolar vs picomolar ranges per gram of brain tissue) (Bortolato et al, 2006; Hohmann et al, 2005).

The role of cannabinoid effects per se on PPI disruption is still a matter of debate. CB_1 receptor agonists were shown to decrease startle amplitude, rendering PPI data difficult for interpretation (Mansbach et al, 1996; Martin et al, 2003). CB_1 agonists were also reported to increase (Stanley-Cary

et al, 2002) or to have no effects on PPI (Bortolato *et al*, 2004), whereas other two studies observed significant impairments of sensorimotor gating (Schneider and Koch, 2002, 2003). Discrepancies between results have been linked to differences in experimental design and/or strain diversity (Bortolato *et al*, 2004). Indeed, clinical data suggest that cannabis use appears to act as a risk factor in the onset of schizophrenia only in individuals who are vulnerable to psychosis, in ways that are still difficult to specify. Therefore, a PPI disruption model based on CB_1 agonists in healthy animals might not capture some of the most relevant gating dysfunctions in schizophrenia, because the experimental subjects might not present any relevant aspects of vulnerability to psychosis. Accordingly, Malone and Taylor (2006) reported that THC produces significant PPI deficits in isolation-reared rats, which already exhibit dysfunctional sensorimotor gating, but not in normal rats. Moreover, genetic CB_1 disruption in mice *per se* does not significantly affect behavior in the social withdrawal model, but highly counteracts the phencyclidine-induced social deficit, which is believed to model negative symptoms of schizophrenia (Haller *et al*, 2005). These results suggest that the relationship between cannabinoid signaling and schizophrenia might be best explored within an experimental context, where CB_1 receptor involvement on the modulation of disturbed major neurotransmitter systems implied in schizophrenia can be investigated.

In the present study, we report that rimonabant and clozapine were equally effective in antagonizing the disruption of PPI produced by phencyclidine. As the PPI-disruptive effects of phencyclidine can be reversed by clozapine and other 'atypical', but not 'typical' antipsychotics in monkeys and rodents (Bakshi *et al*, 1994; Bakshi and Geyer, 1995; Geyer *et al*, 2001; Linn *et al*, 2003; Swerdlow *et al*, 1996), our findings may further strengthen the hypothesis that rimonabant may exhibit an 'atypical' antipsychotic profile. In addition, cotreatment of rimonabant and clozapine significantly reversed PPI deficits induced by phencyclidine, though no augmenting effect was observed, plausibly as both compounds proved fully efficacious in reversing phencyclidine-mediated PPI disruption. Cotreatment might represent a valuable therapeutic option warranting further investigation. Indeed, as rimonabant has been approved for human use as a novel anti-obesity agent, it could perhaps counteract the considerable weight gain induced by clozapine.

Of note, the potent effects of rimonabant in the present investigation on NMDA-induced manipulation of sensorimotor gating were observed with a limited range of doses. This could explain why rimonabant failed to show improvement in global symptom severity or positive and negative symptoms in patients with schizophrenia when compared to placebo in the only published clinical trial to date (Meltzer *et al*, 2004). Other factors, such as the above-suggested differential role of anandamide and 2-AG in psychotogenic mechanisms, as well as the heterogeneity of neurobiological mechanisms underlying psychotic disorders in humans, might contribute to explain the results of Meltzer *et al*.

In conclusion, our results show that rimonabant does mimic the abilities of clozapine to reverse NMDA-induced disruption of PPI. Given that the even more selective CB_1-antagonist AM251 was equally effective in restoring the phencyclidine-induced disruption of PPI, our findings suggest that these effects are mediated by the CB_1 receptor. In the present study, rimonabant also counteracted the PPI disruptive effects in the animal model of deficient sensorimotor gating produced by apomorphine. Insofar as the phencyclidine-PPI model in rats appears to faithfully mimic some aspects of psychosis-related behavior, our findings recommend further interdisciplinary studies to better understand the relationship between glutamate transmission and the role of cannabinoid systems in the pathophysiology of schizophrenia. In addition, our findings recommend further clinical exploration of CB_1 antagonists in selected subpopulations of schizophrenic patients.

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