Cannabidiol, among Other Cannabinoid Drugs, Modulates Prepulse Inhibition of Startle in the SHR Animal Model: Implications for Schizophrenia Pharmacotherapy

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Schizophrenia is a severe psychiatric disorder that involves positive, negative and cognitive symptoms. Prepulse inhibition of startle reflex (PPI) is a paradigm that assesses the sensorimotor gating functioning and is impaired in schizophrenia patients as well as in animal models of this disorder. Recent data point to the participation of the endocannabinoid system in the pathophysiology and pharmacotherapy of schizophrenia. Here, we focus on the effects of cannabinoid drugs on the PPI deficit of animal models of schizophrenia, with greater focus on the SHR (Spontaneously Hypertensive Rats) strain, and on the future prospects resulting from these findings.

Keywords: prepulse inhibition of startle reflex, animal models, schizophrenia, cannabidiol, endocannabinoid system, SHR strain

Schizophrenia is a debilitating neuropsychiatric disorder that affects 0.7% of the world's population (MacDonald and Schulz, 2009) and involves positive (i.e., delusions and hallucinations), negative (e.g., anhedonia, social withdrawal, affective flattening), and cognitive symptoms (such as impaired processing of information and deficits in working memory) (van Os and Kapur, 2009). Currently, schizophrenia's pharmacotherapy is mainly limited to the positive symptoms and associated with severe side effects and high rates of treatment resistance (Briles et al., 2012; Hasan et al., 2012; Abi-Dargham, 2014).

Sensorimotor gating is a physiological process that filters sensory information as it is transmitted to motor output systems, preventing information overload and cognitive...
Prepulse inhibition of startle reflex (PPI) is disrupted in schizophrenia patients and evidence show that PPI deficits are positively correlated to thought disorder (Perry and Braff, 1994; Perry et al., 1999), and associated with impaired functional status and with the presence of auditory hallucinations (Swerdlow et al., 2006; Kumar et al., 2008). PPI deficits are improved by treatment with antipsychotic drugs (Kumari et al., 1999; Weike et al., 2000; Leumann et al., 2002; Oranje et al., 2002; Minassian et al., 2007; Wynn et al., 2007; Martinez-Gras et al., 2009), and this improvement is associated with treatment-related amelioration of schizophrenia symptoms (Minassian et al., 2007). PPI is also disrupted in several animal models of this disorder (Swerdlow et al., 2008), being a useful paradigm to investigate the neurobiology and pharmacotherapy of information processing abnormalities in schizophrenia.

Recent data point to the involvement of the Endocannabinoid system in the pathophysiology of schizophrenia. The endocannabinoid system was described subsequent to the identification of the molecular target of Δ⁹-THC. It comprises the cannabinoid-1 and 2 receptors (CB₁ and CB₂), their endogenous ligands known as endocannabinoids (e.g., anandamide and 2-arachidonoylglycerol), and the enzymes involved in the endocannabinoid's metabolism.

The contribution of the endocannabinoid system in schizophrenia is suggested based on some compelling evidence. The exposure to Δ⁹-THC may induce a transient psychotic condition in healthy subjects (D’Souza et al., 2004; Morrison et al., 2009), whereas in schizophrenia patients, cannabis consumption provokes more and earlier psychotic relapses, even among those under antipsychotic treatment (Linszen et al., 1994; D’Souza et al., 2005; Grech et al., 2005). Moreover, cannabis use has been proved to be a risk factor for psychotic outcomes (Matheson et al., 2011). In accordance, several alterations in the endocannabinoid system are seen in schizophrenia: (1) levels of anandamide are increased in patients’ cerebrospinal fluid and peripheral blood (Leweke et al., 1999, 2007; De Marchi et al., 2003; Giuffrida et al., 2004); (2) post-mortem studies show increased CB₁ density in patients’ dorsolateral prefrontal, anterior cingulate and posterior cingulate cortices (Dean et al., 2001; Zavitsanou et al., 2004; Newell et al., 2006; Dalton et al., 2011); (3) in vivo studies using positron emission tomography (PET) reveal increased density of CB₁ in the brain in both medicated and non-medicated schizophrenia patients (Wong et al., 2010; Ceccarini et al., 2013); (4) polymorphisms of the genes that code the cannabinoid receptors CB₁ and CB₂ are associated to some schizophrenia phenotypes (Ujike et al., 2002; Chavarria-Siles et al., 2008; Ishiguro et al., 2010).

In addition, pre-clinical studies show that cannabinoid drugs are able to modulate schizophrenia-like behaviors, including PPI.

In the work that generated this focused review, we evaluated the effects of four cannabionid drugs on the PPI deficit displayed by the Spontaneously Hypertensive Rat (SHR) strain an animal model characterized by our group to study several aspects of schizophrenia (Levin et al., 2014; Table 1). Here, we will focus on the effects of these four cannabinoids drugs—WIN 55212,2 (WIN—cannabionid agonist), rimonabant (CB₁ antagonist/inverse agonist), AM404 (anandamide uptake

### Table 1: Cannabinoid drugs as potential agents to treat prepulse inhibition of startle (PPI) deficits in schizophrenia.

| Cannabinoid drug | Effect on SHR's PPI | Limitations/advantages |
|------------------|---------------------|------------------------|
| WIN 55212,2      | Attenuates the deficit | Induces psychotomimetic effects in animal models. |
| Rimonabant      | Worsens the deficit  | Is associated with increased symptoms of depression and anxiety. |
| AM 404           | Does not modify PPI  | Displays antipsychotic properties in humans and in other behavioral abnormalities in animal models. Is safe in animals and humans. |
| Cannabidiol      | Attenuates the deficit | |

KEY CONCEPT 4 | Antipsychotic drugs
Drugs used primarily to treat psychotic states, in particular schizophrenia and bipolar disorder. The antipsychotic drugs are classified in typical and atypical compounds: the typical antipsychotic drugs are associated with motor side effects, and the atypical are linked to metabolic disturbances.

KEY CONCEPT 5 | Endocannabinoid system
System described subsequent to the identification of the molecular target of Δ⁹-THC. It comprises the cannabinoid-1 and 2 receptors (CB₁ and CB₂), their endogenous ligands (named endocannabinoids) and the enzymes involved in the endocannabinoid’s metabolism.

fragmentation (Cryan and Reif, 2012). This process is impaired especially in schizophrenia (Braff et al., 2001), but also in other neuropsychiatric disorders such as obsessive-compulsive disorder (Ahmari et al., 2012), Tourette’s syndrome (Swerdlow et al., 2001), Huntington’s disease (Swerdlow et al., 1995), and bipolar disorder (Perry et al., 2001). Prepulse inhibition of startle reflex (PPI) is considered an operational measure of sensorimotor gating and is extensively used in translational studies of schizophrenia, since it is seen in both rodents and humans (Braff et al., 2001). PPI is defined as a reduction of acoustic startle reflex to an intense stimulus (pulse) when immediately preceded by a low intensity stimulus (prepulse).
Inhibitor) and cannabidiol (CB₁ antagonist, anandamide uptake inhibitor, among other effects)—on PPI of animal models, mainly the SHR strain, and on the future prospects resulting from these findings.

SHR strain was developed by selecting rats from Wistar strain with hypertensive phenotype and brother-sister mating (Okamoto and Aoki, 1963). In addition to hypertension, the inbreeding selected some behavioral abnormalities—such as impulsivity, impaired sustained attention and hyperactivity—leading to the proposal of the SHR strain (mainly young animals) as an animal model for attention deficit/hyperactivity disorder (ADHD). It is noteworthy that most of the studies using SHR as ADHD model were performed using as control the Wistar-Kyoto strain (developed by inbreeding Wistar rats without hypertension). Wistar-Kyoto rats may be inappropriate as a control strain since they display inactivity and depressive-like behavior when compared to Wistar rats (WRs) (Overstreet, 2012), and do not show genetic similarities when compared to SHRs (Johnson et al., 1992; St Lezin et al., 1992). In addition, SHRs' predictive validity as ADHD model is inconsistent: several studies describe that the administration of psychostimulants (drugs used to treat ADHD) does not attenuate SHRs' behavioral abnormalities (Amini et al., 2004; Yang et al., 2006; van den Bergh et al., 2006; Bizot et al., 2007; Barron et al., 2009; Calzavara et al., 2009), and may even potentiate them (Amini et al., 2004; Yang et al., 2006; Barron et al., 2009; Calzavara et al., 2009).

Conversely, we reported that SHRs, when compared to WRs, display schizophrenia-like behavioral abnormalities. SHRs display increased locomotion (a model for the positive symptoms of schizophrenia—Lipska and Weinberger, 2000), decreased social interaction (that mimics the negative symptoms—File and Seth, 2003), and deficits in the contextual fear conditioning (associated with impairments in emotional memory seen in schizophrenia—Maren et al., 2013) and PPI. These abnormalities are reversed by antipsychotic drugs (with varied effects in WRs, depending on the drug, the dose, and the behavior), but not by psychostimulant drugs, mood stabilizers, dopaminergic antagonists without antipsychotic activity or drugs that modulate anxiety (Calzavara et al., 2009, 2011; Levin et al., 2011). In addition, psychotomimetic manipulations, such as sleep deprivation and administration of psychostimulants, potentiate the behavioral abnormalities displayed by SHRs and induce a schizophrenia-like behavioral phenotype in WRs (Calzavara et al., 2009, 2011; Levin et al., 2011)—as seen in other animal models of the disorder, as well as in patients (Laruelle et al., 1999; Jones et al., 2011). As a result, the SHR strain has been used to investigate genetic alterations related to schizophrenia as well as novel therapeutic strategies for this disorder, including cannabinoid drugs (Levin et al., 2012, 2014; Almeida et al., 2013, 2014; Diana et al., 2015).

WIN is an agonist of CB₁ and CB₂ receptors. The acute administration of WIN attenuates the SHRs' PPI deficit, and does not modify the PPI of WRs—although a trend to reduce the WRs' PPI is seen with the lowest dose (Levin et al., 2014). Accordingly, other studies demonstrate that WIN reverses the PPI impairment displayed by psychosocially stressed mice (Brzózka et al., 2011) and induced by chronic administration phenycyclidine (Spano et al., 2010), two other animal models of schizophrenia. The beneficial effect of WIN on the PPI of psychosocially stressed mice is prevented by pretreatment with the CB₁ antagonist rimonabant (Brzózka et al., 2011), suggesting an involvement of these receptors on the WIN's actions on sensorimotor gating functioning.

In rodents without PPI deficits some authors describe absence of WIN effect on PPI (Bortolato et al., 2005; Brzózka et al., 2011), but others show that WIN disrupts PPI when administered systemically (Schneider and Koch, 2002; Wegener et al., 2008; Brosda et al., 2011), intra-prefrontal cortex, or intra-ventral hippocampus (Wegener et al., 2008). In addition, repeated administration of WIN during puberty induces PPI deficits that last until adulthood (Schneider and Koch, 2003; Schneider et al., 2005; Wegener and Koch, 2009; Klein et al., 2013). Therefore, WIN seems to present a psychotomimetic profile (supported also by its effects on other schizophrenia-like symptoms—Schneider and Koch, 2002; Pamplona and Takahashi, 2006; Wegener et al., 2008; Spano et al., 2010; Almeida et al., 2014), being unsuitable for schizophrenia patients.

The administration of the CB₁ antagonist rimonabant worsens SHRs' PPI deficit, and does not alter PPI levels in WRs (Levin et al., 2014). The absence of rimonabant effects in WRs is corroborated by previous studies with animals without PPI impairments (Martin et al., 2003; Malone et al., 2004; Malone and Taylor, 2006; Ballmaier et al., 2007). In animal models of schizophrenia, while some studies show that rimonabant is able to counteract the PPI impairments (Malone et al., 2004; Nagai et al., 2006; Ballmaier et al., 2007), others show no effect (Martin et al., 2003; Malone and Taylor, 2006). In addition, clinical trials show that rimonabant induces significant psychiatric side effects, such as symptoms of depression and anxiety, and increases suicide-related adverse events (Christensen et al., 2007; Food and Drug Administration Advisory Committee, 2007; Topol et al., 2010). These data, thus, argue against the clinical use of this compound.

It should be noted that both a CB₁/CB₂ agonist and a CB₁ antagonist provoke differential effects in WRs and SHRs (Levin et al., 2014). This information suggests that these rat strains display distinct endocannabinoid system functioning. Indeed, our group observed that SHRs present higher CB₁ density in the prefrontal and anterior cingulate cortices when compared to WRs (Almeida et al., submitted), which is in accordance with data from schizophrenia patients (Dean et al., 2001; Zavitsanou et al., 2004; Newell et al., 2006; Wong et al., 2010; Dalton et al., 2011; Ceccarini et al., 2013).

AM 404 is a competitive and selective inhibitor of anandamide transportation, and therefore increases its extracellular levels. Anandamide is an endocannabinoid that acts as an agonist of CB₁ and CB₂ receptors and of vanilloid receptor 1 (TRPV1).
When administered to WRs and SHRs, AM 404 did not modify their PPI levels (Levin et al., 2014). No other study has investigated the effects of AM 404 on PPI of animal models of schizophrenia. In control animals, one study shows absence of AM 404 effects on PPI in Sprague-Dawley rats (Bortolato et al., 2006), but another reveals that AM 404, either injected acutely or chronically, disrupts PPI in Swiss mice (Fernandez-Espejo and Galan-Rodriguez, 2004). Evidence, therefore, do not support the

| Reference | Results |
|-----------|---------|
| Schneider and Koch, 2002 | Acute administration of WIN (0.6 or 1.2 mg/kg) impairs PPI in a dose-dependent manner. The administration of haloperidol reverses the PPI deficit. |
| Schneider and Koch, 2003; Schneider et al., 2005 | Treatment for 25 days with WIN (1.2 mg/kg) during puberty induces PPI deficits that last until adulthood. This impairment is reversed by the administration of haloperidol. |
| Bortolato et al., 2005 | Chronic (during 7 or 21 days) or acute treatment with WIN (0.5, 1, or 2 mg/kg) does not alter PPI levels. |
| Wegener et al., 2008 | Acute systemic administration of WIN (1.2 mg/kg), as well as the administration intra-medial prefrontal cortex or intra-dorsal hippocampus (5 µg/0.3 µl) diminish PPI levels. |
| Wegener and Koch, 2009 | Treatment for 25 days with WIN (1.2 mg/kg) during puberty induces PPI deficits that last until adulthood. In addition, WIN treated animals display altered basal neuronal activity and respond differently to haloperidol and apomorphine. |
| Spano et al., 2010 | Chronic WIN self-administration (12.5 µg/kg/infusion) as well as experimenter-given (0.3 mg/kg, i.v.) attenuates phencyclidine-induced impairments in PPI. |
| Brosda et al., 2011 | Acute systemic administration of WIN (0.6 or 1.2 mg/kg) impairs PPI. |
| Brzózka et al., 2011 | WIN (3 mg/kg) administration restores the PPI deficit induced by chronic psychosocial stress. This effect is antagonized by pretreatment with rimonabant. |
| Klein et al., 2013 | Chronic treatment with WIN (1.2 mg/kg) during puberty induces PPI deficits that last until adulthood and are reversed by deep brain stimulation. |
| Levin et al., 2014 | Acute administration of WIN (1 mg/kg) restores the PPI deficit displayed by the SHR strain. WIN (0.3, 1 or 3 mg/kg) does not alter the PPI of control animals. |
| Martin et al., 2003 | Acute administration of rimonabant (5 mg/kg) does not alter PPI on its own or following disruptions by apomorphine, d-amphetamine or MK-801. |
| Malone and Taylor, 2006 | Acute administration of rimonabant (5 mg/kg) is not able to counteract the PPI deficit promoted by social isolation. In addition, rimonabant does not alter the PPI of control animals. |
| Nagai et al., 2006 | Acute administration of rimonabant (10 mg/kg) reverses the ∆9-THC-induced PPI deficit and increased dopamine release in the nucleus accumbens. |
| Ballmaier et al., 2007 | Acute administration of rimonabant (0.75, 1.5, or 3.0 mg/kg) does not alter PPI on its own, and counteracts the PPI disruption induced by administration of phencyclidine, MK-801 or apomorphine. |
| Levin et al., 2014 | Acute administration of rimonabant (0.75 mg/kg) worsens the PPI deficit displayed by the SHR strain. Rimonabant (0.75, 1.5, or 3 mg/kg) does not alter the PPI of control animals. |
| Fernandez-Espejo and Galan-Rodriguez, 2004 | AM 404 either injected acutely (2.5 mg/kg) or chronically (5 mg/kg daily, 7 days) disrupts PPI. This effect is blocked by pretreatment with rimonabant. |
| Bortolato et al., 2006 | Acute administration of AM 404 (2.5, 5, or 10 mg/kg) does not alter PPI levels. |
| Levin et al., 2014 | Acute administration of AM 404 (1, 5, or 10 mg/kg) does not alter the PPI of SHRs or Wistar rats. |
| Long et al., 2006 | Acute administration of cannabidiol (1, 5, or 15 mg/kg) does not alter PPI on its own, but reverses (5 mg/kg) the MK-801-induced disruption of PPI. Pretreatment with capsazepine (agonist of TRPV1 receptors) prevents cannabidiol effect. |
| Gururajan et al., 2011 | Acute administration of cannabidiol (10, 30, or 50 mg/kg) disrupts PPI on its own, and has no effect on MK-801-induced PPI disruption. |
| Levin et al., 2014 | Acute administration of cannabidiol (30 mg/kg) restores the PPI deficit displayed by the SHR strain. Cannabidiol administration also increases the PPI levels of control animals. |
| Gomes et al., 2015 | Treatment with MK-801 for 28 days impairs PPI. Chronic treatment with cannabidiol (30 or 60 mg/kg) attenuates this impairment. Cannabidiol does not alter PPI on its own. |
| Pedrauzzi et al., 2015 | Pretreatment with cannabidiol (15, 30, or 60 mg/kg) attenuates the amphetamine-induced disruption of PPI. Cannabidiol does not alter PPI on its own. |

MK-801, dizocilpine; PPI, prepulse inhibition of startle; SHR, spontaneously hypertensive rats; ∆9-THC, delta-9-tetrahydrocannabinol; WIN, WIN 55212,2.
Acute administration of cannabidiol (60 mg/kg) diminishes the stereotyped behavior induced by apomorphine, without promoting catalepsy. Pretreatment with cannabidiol (3 mg/kg) counteracts the hyperlocomotion and the decrease in social interaction induced by MK-801. When administered to rats, acute or chronic administration of cannabidiol (50 mg/kg, 21 days) attenuates the dexamphetamine-induced hyperlocomotion. This effect is mainly seen when PPI levels are below 50%, which is our case. Therefore, the effects of cannabidiol on PPI of WRs are in accordance with the antipsychotic profile suggested for this drug.

The mechanisms whereby cannabidiol modifies PPI are still to be elucidated. Our data suggest that the increase in PPI promoted by this drug is not only due to an increase in anandamide levels, since the anandamide uptake inhibitor AM 404 does not modify PPI (Gomes et al., 2015). Acute or chronic administration of psychostimulant drugs such as MK-801 and amphetamine are used to model schizophrenia. The results, thus, point to an antipsychotic profile of cannabidiol and to the use of this compound on the treatment of sensorimotor gating impairments seen in schizophrenia.

In animals without PPI impairments, some authors describe that cannabidiol does not modify the PPI levels when administered acutely or chronically (Long et al., 2006; Gomes et al., 2015; Pedrazzi et al., 2015), while one study shows that it is able to disrupt PPI (Gururajan et al., 2011). Nonetheless, in our previous work, administration of cannabidiol increased PPI in WRs (Levin et al., 2014). Interestingly, typical and atypical antipsychotic drugs have been shown to increase PPI in WRs and Sprague-Dawley rats (Hoffman et al., 1993; Swerdlow and Geyer, 1993; Johansson et al., 1995; Depoortere et al., 1997a,b; Levin et al., 2011). This effect is mainly seen when PPI levels are below 50%, which is our case. Therefore, the effects of cannabidiol on PPI of WRs are in accordance with the antipsychotic profile suggested for this drug.

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the Ki for displacement of [3H]CP55940 from CB1 and CB2 (Pertwee, 2008). In addition, the effectiveness of cannabidiol in inhibiting [35S]GTP/Y binding to brain membranes of wild-type mouse is not different from CB1−/− mouse (Pertwee, 2008). On the other hand, Long et al. (2006) described that cannabidiol’s ability of restoring the PPI deficit induced by MK-801 is prevented by pretreatment with capsaicin, a TRPV1 antagonist. Thus, although other mechanisms of action cannot be disregarded, cannabidiol’s effects on PPI seem to be at least partially related to its action on the vanilloid system.

Other animal studies, investigating additional schizophreniaklike behavioral paradigms, support cannabidiol’s antipsychotic properties (Table 3). The administration of this compound is able to diminish the stereotyped behavior and the hyperlocomotion—behaviors that model the positive symptoms of schizophrenia—induced by psychotomimetic drugs (Zuardi et al., 1991; Moreira and Guimaraes, 2005; Long et al., 2010; Gururajan et al., 2012). Cannabidiol also restores drug-induced impairments in social interaction (Malone et al., 2009; Gururajan et al., 2012), and counteracts the deficit in contextual fear conditioning displayed by the SHRs (Levin et al., 2012).

The promising aforementioned pre-clinical data led to clinical studies (Table 3). In healthy volunteers, cannabidiol is able to attenuate the psychotic symptoms induced by the administration of psychotomimetic drugs (Karniol et al., 1974; Zuardi et al., 1982; Bhattacharyya et al., 2010). In a case-study, this cannabinoid significantly reduced schizophrenia symptoms in the Brief Psychiatric Rating Scale (BPRS), in a way superior to the typical antipsychotic drug haloperidol (Zuardi et al., 1995). Moreover, an open-label study with Parkinson’s disease patients showed that cannabidiol administration, in addition to their usual treatment, decreases psychotic symptoms—evaluated by BPRS and by the Parkinson Psychosis Questionnaire—without worsening their motor function (Zuardi et al., 2009). Also, several studies suggest that cannabidiol is safe in humans and animals, and does not alter physiological parameters (blood pressure, heart rate and body temperature) or induce motor and psychological side effects (Bergamaschi et al., 2011).

Thereafter, a double-blind controlled clinical trial with schizophrenia patients was performed: treatment with cannabidiol, for 4 weeks, decreased patients symptoms—evaluated by BPRS and Positive and Negative Syndrome Scale (PANSS)—in a way non-inferior to amisulpride, one of the most effective antipsychotic drugs currently in use. Interestingly, the cannabidiol induced fewer side effects (weight gain, changes in prolactin levels and extrapyramidal symptoms) than amisulpride, and treatment with cannabidiol, but not with amisulpride, led to an increase in the levels of anandamide in serum that was associated with the decrease of psychotic symptoms (Leweke et al., 2012).

All these encouraging clinical and pre-clinical data led to a recent proof-of-concept study by GW Pharmaceuticals. The phase IIa included 88 schizophrenia patients only partially responsive to standard antipsychotic treatment, who received cannabidiol or placebo in addition to their antipsychotic medication for 6 weeks. Cannabidiol was consistently superior to placebo in attenuating the schizophrenia symptoms, and did not induce serious adverse events (GW Pharmaceuticals, 2015).

Taken as a whole, data regarding the effects of cannabidiol drugs on PPI reinforce the involvement of the endocannabinoid system in the sensorimotor gating functioning and in the pathophysiology of schizophrenia. Among the drugs that act on the endocannabinoid system, pre-clinical and the subsequent clinical data point to cannabidiol as the most promising compound for treating schizophrenia symptoms without inducing significant side effects. Nevertheless, most of the clinical evidence that suggests cannabidiol as a new antipsychotic agent or adjunctive treatment does not investigate specifically sensorimotor gating/ PPI deficits. Accordingly, data from pre-clinical studies using animal models, like our previous one (Levin et al., 2014), are fundamental to support future clinical studies focused on these deficits in schizophrenia patients.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest Statement: JH, AZ, and JC are co-inventors of the patent "Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023."

The Other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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