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Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test

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ABSTRACT

Cannabidiol (CBD), a non-psychotomimetic compound of the *Cannabis sativa*, has been reported to have central therapeutic actions, such as antipsychotic and anxiolytic effects. We have recently reported that Spontaneously Hypertensive Rats (SHRs) present a deficit in social interaction that is ameliorated by atypical antipsychotics. In addition, SHRs present a hyperlocomotion that is reverted by typical and atypical antipsychotics, suggesting that this strain could be useful to study negative symptoms (modeled by a decrease in social interaction) and positive symptoms (modeled by hyperlocomotion) of schizophrenia as well as the effects of potential antipsychotics drugs. At the same time, an increase in social interaction in control animals similar to that induced by benzodiazepines is used to screen potential anxiolytic drugs. The aim of this study was to investigate the effects of CBD on social interaction presented by control animals (Wistar) and SHRs. The lowest dose of CBD (1 mg/kg) increased passive and total social interaction of Wistar rats. However, the hyperlocomotion and the deficit in social interaction displayed by SHRs were not altered by any dose of CBD. Our results do not support an antipsychotic property of cannabidiol on symptoms-like behaviors in SHRs but reinforce the anxiolytic profile of this compound in control rats.

1. Introduction

Cannabidiol (CBD), one of the major constituents of *Cannabis sativa* (Crippa et al., 2010; Mechoulam et al., 1970; Zuardi, 2008), was initially believed to lack psychoactive actions. Nevertheless, studies have shown that CBD produces effects on the central nervous system. Initial observation in healthy volunteers showed that CBD reverses the anxiogenic and psychotomimetic effects induced by Δ9-THC, the main psychoactive compound of *C. sativa* (Karniol et al., 1974; Zuardi et al., 1982, 2010).

Further studies have described anxiolytic properties of this compound in rodents (Campos and Guimaraes, 2008; Guimaraes et al., 1990; Moreira et al., 2006, 2009; Resstel et al., 2006) and in humans (Bergamaschi et al., 2011; Crippa et al., 2011; Zuardi et al., 1993). CBD has also been suggested as a potential antipsychotic drug (see Zuardi et al., 2012, for review). This suggestion is based on animal (Long et al., 2012; Moreira and Guimaraes, 2005; Zuardi et al., 1991) and clinical (Leweke et al., 2000, 2012; Zuardi et al., 1995, 2006a,b) studies indicating that CBD exhibits a behavioral and neurochemical profile that resembles that of atypical antipsychotics. These studies demonstrate that this compound attenuates symptoms of schizophrenia without inducing extrapyramidal side effects (Crippa et al., 2010; Cunha et al., 1980; Moreira and Guimaraes, 2005; Zuardi et al., 1991, 1995, 2006b). In addition, CBD was able to increase the number of Fos immunoreactive neurons similar to that of an atypical antipsychotic (Guimaraes et al., 2004).

Based on its predictive and face validities, a decrease in social interaction has been described in several animal models of schizophrenia as a behavioral parameter that mirrors the negative symptoms of this disease (O'Tuathaigh et al., 2010; Sams-Dodd, 1995, 1998a,b,c; Sams-Dodd et al., 1997). In this test, locomotion – which increase has been suggested to model positive symptoms of schizophrenia based on its predictive and construct validities (Lipska and Weinberger, 2000; Powell and Miyakawa, 2006; Van den Buuse et al., 2005) – and rearing frequency – considered to represent a nonsocial exploratory behavior in a social situation (Ando et al., 2006; O'Tuathaigh et al., 2008, 2010) – can also be quantified. Recently, our group has suggested the SHR (Spontaneously Hypertensive Rats) strain as a good animal model to study several aspects of schizophrenia (Calzavara et al., 2009, 2011a,b; Levin et al., 2011).

Abbreviations: CBD, cannabidiol; SHR, Spontaneously Hypertensive Rats; WR, Wistar rats.

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Particularly, this strain presents a decrease in social performance that is attenuated by atypical antipsychotics while its increase in locomotion is diminished by typical and atypical antipsychotics (Calzavara et al., 2011a), reinforcing its predictive validity as an animal model to investigate positive and negative symptoms of schizophrenia.

In parallel, levels of social interaction in rodents have been used to evaluate pharmacological effects on anxiety. An increase in social interaction is indicative of an anxiolytic effect, whereas a decrease is induced by anxiogenic drugs (File and Hyde, 1978; File and Seth, 2003).

Considering the potential anxiolytic and antipsychotic action of CBD, the aim of the present study is to investigate the effects of several doses of this compound on Wistar rats (control animals) and SHR (animal model of schizophrenia) evaluated in the social interaction test. In this sense, we expect that as an anxiolytic drug, CBD would increase social interaction in control animals whereas its antipsychotic profile would be revealed by an increase in social performance and decrease in hyperlocomotion in SHR.

2. Material and methods

2.1. Animals

Male adult Wistar rats (WR) and SHR (five-month-old) from our own colony were housed under conditions of controlled temperature (22–23 °C) and lighting (12/12 h light/dark cycle, lights on at 07:00 am). Groups of 5 animals were kept in Plexiglas cages (41×34×16.5 cm), with free access to food and water. Animals were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. This study was approved by the Ethical Committee of Federal University of Sao Paulo. Rats were used only once and were drug-naive before each experiment.

Although Wistar Kyoto (WKY) is the background strain for SHR and in contrast to several other researchers who use WKY rats as controls, we chose to use WR in this work, as in our previous study (Calzavara et al., 2009). The use of WKY rats as normotensive controls for SHR has been questioned because of the remarkable behavioral differences of WKY rats when compared to other normotensive strains (Diana, 2002; Drolet et al., 2002; Hård et al., 1985; Paré, 1994). Indeed, the reported increase in locomotor activity in SHRs may, in fact, be a consequence of the well-demonstrated hypomotility of WKYs (Dugovic et al., 2000; Hård et al., 1985; McCarty and Kirby, 1982; Rosecrans and Adams, 1976; Tilson et al., 1977). In addition, there is evidence that WKY rats display a high level of anxiety-like behavior and score high on depressive behaviors in many models of depression (Dugovic et al., 2000; Paré, 1992a,b, 1993, 1994).

2.2. Drugs

CBD (kindly supplied by THC Pharm, Germany and STI-Pharm, Brentwood, UK) was suspended in polyoxyethylenesorbitan monooleate (Tween 80) 2% in saline 0.9%. Saline plus Tween 80 were used as control solution. All solutions were intraperitoneally injected (i.p.) in a volume of 1 ml/kg body weight.

2.3. Social interaction test

The social interaction (SI) test was performed in accordance with previous studies (Calzavara et al., 2011a; O'Tuathaigh et al., 2010; Sams-Dodd, 1998b). The test was conducted in an open-field arena (97 cm in diameter and 32.5 cm high, with an open top and a floor divided into 19 similar parts) between 8 and 11 am. Pairs of unfamiliar rats of the same treatment and strain were placed simultaneously into the unfamiliar apparatus approximately 80 cm apart. Social behaviors and locomotor activity parameters were scored live for 10 min. Time spent in active (sniffing and following) or passive (when animals lie next to each other within a distance of 5 cm from skin to skin) was scored for each rat. The total SI time was calculated by the sum of the time spent in active and passive social behaviors. Although some data report that the pair of rats should be treated as one point (File and Seth, 2003), the present study quantified the social behaviors for each rat. This procedure was conducted based on the fact that active interaction reflects the motivation to interact and does not necessarily reflect the behavior of both animals, while passive interaction depends on the pair. Additionally, the evaluation of active and passive interaction separately and for each animal of the pair allow the investigation of social behaviors between animals of different strains or drug treatments, increasing the repertoire of information originated from this behavioral task (Calzavara et al., 2011a; Long et al., 2010, 2012). Locomotor activity (number of floor squares entered) and rearing frequency (the number of times each animal stood on its hind legs without interacting with a partner) were also recorded. The observers were blind to the treatment and strain of the rats.

2.4. Experimental design

2.4.1. Experiment 1 — effects of high doses of cannabidiol on social interaction, rearing frequency and locomotor activity of WRs and SHRs

WRs and SHRs (n = 10/strain/drug treatment) were treated with vehicle, 15, 30 or 60 mg/kg CBD. Thirty minutes after the injection, the animals were submitted to the social interaction test.

2.4.2. Experiment 2 — effects of lower doses of cannabidiol on social interaction, rearing frequency and locomotor activity of WRs and SHRs

WRs and SHRs (n = 10–12/strain/drug treatment) were treated with vehicle, 1, 5 or 15 mg/kg CBD. Thirty minutes after the injection, the animals were submitted to the social interaction test.

Doses and schedules were chosen based on previous studies (Moreira and Guimaraes, 2005; Zuardi et al., 1991).

2.5. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) with treatment and strain as between-subjects factors followed by Duncan's post-hoc test when applicable. A significance threshold of $p<0.05$ was used.

3. Results

3.1. Experiment 1 — effects of high doses of cannabidiol on social interaction, rearing frequency and locomotor activity of WRs and SHRs

For total social interaction time (Fig. 1a), active and passive interaction (Table 1), two way ANOVA revealed a significant strain effect [$F(1,72) = 54.48, 9.90, 43.07$; $p<0.05$, respectively]. SHRs presented a decrease in social interaction time when compared to WR.

For locomotion frequency (Fig. 1b), two-way ANOVA revealed a significant strain effect [$F(1,72) = 18.03$; $p<0.05$]. SHRs presented higher locomotion frequency when compared to WR rats.

For rearing frequency (Fig. 1c), two-way ANOVA revealed a significant strain effect [$F(1,72) = 100.25$; $p<0.05$]. Rearing frequencies of SHRs were higher than that of WRs.

The lack of a significant drug effect indicates that there was no effect of CBD treatment on all parameters observed independently of the strain.

3.2. Experiment 2 — effects of lower doses of cannabidiol on social interaction, rearing frequency and locomotor activity of WRs and SHRs

Two way ANOVA revealed a significant strain effect for total social interaction time (Fig. 2a), active and passive interaction (Table 1) [$F(1,86) = 131.56, 14.50, 113.39$; $p<0.05$, respectively], and an interaction between strain and treatment factors for total social interaction

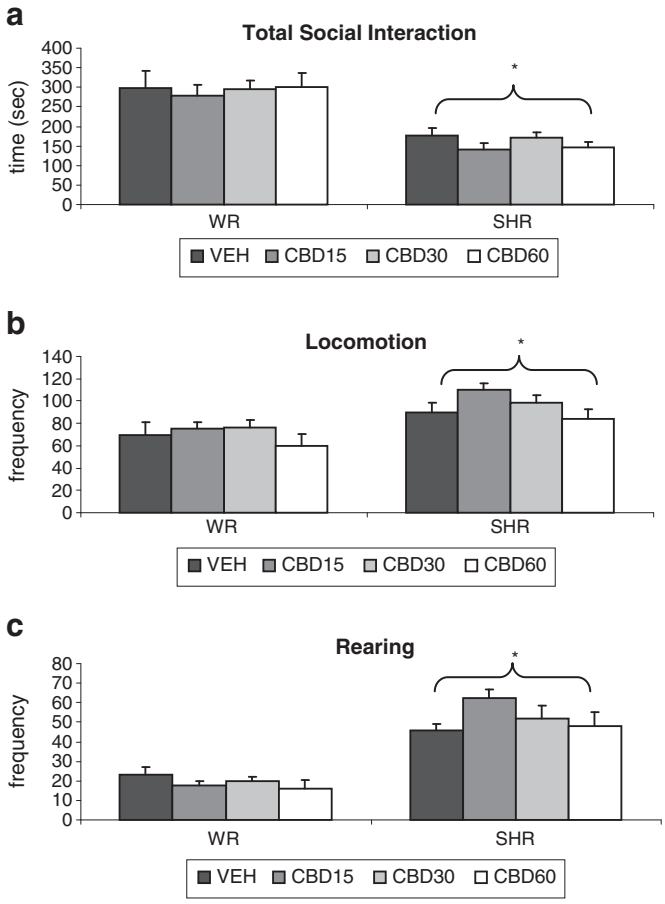

Fig. 1. a) Total social interaction (active and passive), b) locomotion frequency and c) rearing frequency of WR and SHR treated with vehicle (VEH) or 15, 30 or 60 mg/kg cannabidiol (CBD). * $p<0.05$ compared to WRs.

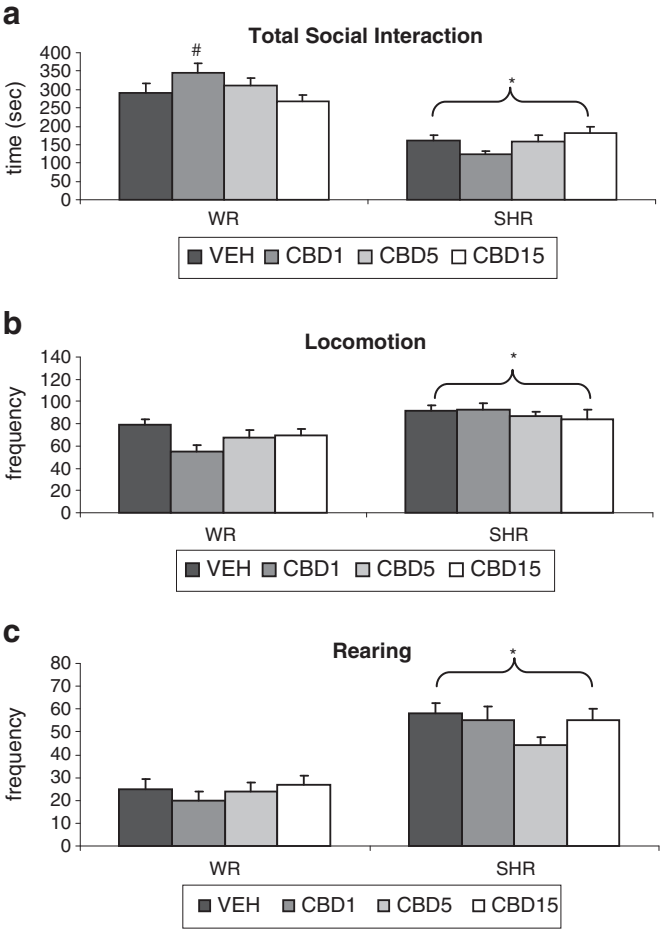

Fig. 2. a) Total social interaction (active and passive), b) locomotion frequency and c) rearing frequency of WR and SHR treated with vehicle (VEH) or 1, 5 or 15 mg/kg cannabidiol (CBD). * $p<0.05$ compared to WR; # $p<0.05$ compared to VEH group.

(Fig. 2a) and passive interaction (Table 1) [$F(3,86)=4.76, 5.01$; $p<0.05$, respectively]. SHRs presented a decrease in social interaction time when compared to WRs. Duncan post-hoc analysis revealed that CBD (1 mg/kg) increased total social interaction and passive interaction in WRs, but not in SHR.

For locomotion frequency (Fig. 2c), two-way ANOVA revealed a significant strain effect [$F(1,86)=24.41$; $p<0.05$]. SHRs presented higher locomotion frequency when compared to WR rats.

For rearing frequency (Fig. 2c), two-way ANOVA revealed a significant strain effect [$F(1,86)=87.71$; $p<0.05$]. Rearing frequencies of SHRs were higher than that of WRs.

Additionally, a Student T-Test was performed between the vehicle-treated groups of both strains to confirm the decrease in social interaction presented by SHR animals ($t(22)=2.770, 4.083, 4.970$, $p<0.05$ for active, passive and total social interaction, respectively).

4. Discussion

The present study showed that acute treatment with CBD did not reverse the deficit in social interaction (decreased time spent in total, passive and active social interactions) nor the elevated locomotion and rearing frequency of SHR. However, it was observed that a low acute dose of CBD (1 mg/kg) increased passive and total social interaction in WRs.

As commented, the social interaction test has been used to study social aspects of schizophrenia in several different animal models,

Table 1
Active and passive social interactions of WR and SHR. Data are reported as mean ± SE. *$p<0.05$ compared to WR, # $p<0.05$ compared to VEH group of the same strain.

	Treatment	Active social interaction (s)			Passive social interaction (s)		
		Strain					
		WR	SHR		WR	SHR	
Experiment 1	VEH	24.8 ± 10.0	7.6 ± 2.0	*	274.3 ± 48.4	169.4 ± 18.1	*
	CBD15	18.6 ± 4.7	6.7 ± 1.3		259.7 ± 26.6	134.4 ± 15.8	
	CBD30	13.0 ± 2.8	5.0 ± 1.1		282.3 ± 22.8	164.7 ± 15.0	
	CBD60	7.4 ± 1.3	6.7 ± 2.3		294.2 ± 35.9	140.8 ± 12.6	
Experiment 2	VEH	26.2 ± 9.4	5.9 ± 1.7	*	268.7 ± 22.3	155.8 ± 13.3	*
	CBD1	22.2 ± 10.5	4.9 ± 1.0		323.8 ± 23.5#	119.7 ± 6.3	
	CBD5	21.2 ± 6.9	7.2 ± 2.3		288.2 ± 22.9	151.7 ± 10.2	
	CBD15	21.0 ± 5.6	6.2 ± 1.4		247.1 ± 14.3	175.6 ± 17.7	

and impairments in social performance in this test have been suggested to model the negative symptoms of the disease (O'Tuathaigh et al., 2010; Sams-Dodd, 1995, 1998a,b,c; Sams-Dodd et al., 1997). A previous study of our laboratory (Calzavara et al., 2011a) showed that SHRs exhibit decreases in social interaction (passive, active and total) and increases in rearing frequency when compared to control animals (as observed in the present study). These behavioral alterations were ameliorated only by atypical antipsychotics (Calzavara et al., 2011a), in accordance with the clinical effectiveness of these drugs on the negative symptoms of schizophrenia (Miyamoto et al., 2005). Unlike atypical antipsychotics, we observed that acute treatment of CBD had no effect on the impairment in social performance presented by SHR.

The antipsychotic effect and the atypical profile of CBD have been suggested in previous clinical and animal studies. These studies revealed that CBD can reduce behaviors that resemble positive symptoms of schizophrenia without inducing extrapyramidal side effects, a profile characteristic of atypical drugs. The first investigation of antipsychotic properties in animal models of positive symptoms showed that, similar to the typical antipsychotic haloperidol, acute CBD reduced apomorphine-induced stereotyped behavior but without inducing catalepsy (opposite to haloperidol) even at high doses (Zuardi et al., 1991). In agreement, acute treatment with CBD reversed hyperlocomotion induced by D-amphetamine and ketamine in mice, an effect also observed with haloperidol and the atypical antipsychotic clozapine (Moreira and Guimaraes, 2005). However, only clozapine and CBD did not cause catalepsy. In addition, brain structures such as nucleus accumbens and dorsal striatum have been proposed to mediate the effect of antipsychotic drugs on the positive symptoms and motor side effects, respectively (Seeman, 2002; Strange, 2001). A study employing Fos protein to detect neuronal activation showed that CBD, like clozapine, activates Fos-expression in the nucleus accumbens, while haloperidol activates the nucleus accumbens and dorsal striatum (Guimaraes et al., 2004). However, brain structures that can be involved with negative symptoms, such as prefrontal cortex, were not investigated in the same study.

Previous studies described the effects of CBD on models of negative symptoms: this compound was able to attenuate the deficit in social interaction induced by MK-801 (Gururajan et al., 2011) when acutely administered. In addition, Long et al. (2012) showed that long-term CBD enhanced the social interaction of *neuregulin 1* mutant mice (a putative animal model of schizophrenia). The discrepancies of these studies and the present results could be explained by differences in the protocols used, as the animal model and the dose schedule (acute X long-term treatment). Possibly, a long-term CBD treatment would be necessary to reverse or improve the deficit in social interaction presented by SHR.

The antipsychotic effect of CBD was also investigated in humans. These data reported a significant improvement of the general symptoms detected by the Brief Psychiatric Rating Scale (BPRS) of a female patient with schizophrenia who was treated with increasing doses of CBD (Zuardi et al., 1995). The same group further showed that CBD therapy in treatment-resistant schizophrenia patients was mildly effective for both in positive and negative symptoms (Zuardi et al., 2006b). Additionally, the first double-blind controlled clinical trial demonstrated that chronic treatment with CBD, similar to the atypical antipsychotic amisulpride, reduced psychotic symptoms in schizophrenia patients (Leweke et al., 2012). These clinical studies showed that CBD was well tolerated and no side effects were reported.

In contrast to the putative antipsychotic effects of CBD on positive symptoms of schizophrenia, the present results demonstrate that acute CBD was not able to reverse the hyperlocomotion presented by SHR. As previously mentioned, the increase in locomotion has been proposed as a model of positive symptoms of schizophrenia (Calzavara et al., 2011a; Lipska and Weinberger, 2000; Van den Buuse and de Jong, 1989). In this context, we have previously reported that the hyperactivity of SHR was ameliorated by typical and atypical antipsychotic (Calzavara et al., 2011a), which reflects the action of these drugs for positive symptoms of schizophrenia (Bouchard et al., 2000). In accordance with the absence of acute effects of CBD on the hyperlocomotion displayed by SHR, a large range dose of CBD was not able to reverse dexamphetamine-induced hyperlocomotion in C57BL/6JArc mice (Long et al., 2010). In addition, another study showed that CBD was not able to reverse dexamphetamine hyperlocomotion in animal model of mania (Valvassori et al., 2009). Possibly, the differences between the present result and those showing that CBD reduced psychostimulant-induced hyperlocomotion (Moreira and Guimaraes, 2005) could be related to the different strains and species used. In parallel, it should be noted that an important difference between these and our present study is that hyperlocomotion presented by SHRs is spontaneous. In accordance, a recent study showed that spontaneous hyperlocomotion of *neuregulin 1* mutant mice was not reversed by acute or long-term CBD (Long et al., 2012).

Although the analyses of acute CBD on negative (modeled by deficit on social interaction) and positive (modeled by hyperlocomotion) symptoms in SHR do not indicate an antipsychotic effect, we have recently described that acute CBD reverses the deficit in contextual fear conditioning displayed by SHR (Levin et al., 2012), a behavioral impairment related to emotional processing deficits in schizophrenia (Calzavara et al., 2009, 2011b). Considering a recent clinical study reporting that chronic CBD exerts relevant antipsychotic effects on negative and positive symptoms of schizophrenic patients (Leweke et al., 2012), a possible beneficial effect of a chronic treatment on the social interaction deficit and hyperlocomotion displayed by SHR cannot be ruled out and merits further investigation.

Conversely, the lowest acute dose of CBD was able to increase social interaction in Wistar rats, an effect that can be related to an anxiolytic property of this drug. In this sense, anxiolytic properties of CBD have been reported in several studies with rodents using different paradigms such as conditioned fear (Resstel et al., 2006), elevated plus-maze (Campos and Guimaraes, 2008; Guimaraes et al., 1990, 1994; Moreira et al., 2009), prey vs predator paradigm (Uribe-Mariño et al., 2012) and Vogel conflict test (Moreira et al., 2006). The anxiolytic effect of CBD was also evaluated in humans. Simulated public speaking is a test that can induce anxiety and is sensitive to the effects of anxiolytic drugs. In this test, CBD had an anxiolytic effect similar to that of diazepam and ipsapirone in healthy subjects (Zuardi et al., 1993). In the same way, CBD had an anxiolytic effect on patients with generalized social anxiety disorder submitted to simulated public speaking (Bergamaschi et al., 2011), an effect that seems to be mediated by activation of limbic and paralimbic brain regions (Crippa et al., 2011).

Of note, CBD enhances the passive but not the active social interaction. Passive and active social interactions seem to reflect different social behaviors. As described previously (Calzavara et al., 2011a), Wistar rats placed together with an unfamiliar rat typically perform an initial inspection of the arena and the unfamiliar rat. After this initial inspection, the two rats interact frequently while exploring and moving around the arena together. The interaction can be active (sniffing and following) or passive (the animals lie next to each other). The active social interaction could be related to a behavior mediated by motivational drive to interact because the rat is actively looking for and investigating the unfamiliar rat. On the other hand, passive interaction predicts that the rodent accepts the presence of an unfamiliar rat. In this context, it could be suggested that the alleged anxiolytic effect of CBD treatment enhances the acceptance of an unfamiliar rat, but not the motivational drive to interact.

The molecular mechanisms of the anxiolytic action of CBD remain unknown. While the anxiolytic effects of CBD are similar to those induced by diazepam, the role of benzodiazepine receptors in the anxiolytic property of CBD is controversial (Moreira et al., 2006; Onaivi et al., 1990). In this respect, studies have shown that CBD binds to CB1 receptor with low affinity (Petitet et al., 1998; Thomas et al., 1998), activates vaniloid receptors, inhibits the cellular uptake and hydrolysis of anandamide (Bisogno et al., 2001) and acts as agonist in

the 5HT1A receptors (Russo et al., 2005). In accordance with other cannabinoid compounds, a biphasic effect of CBD in anxiety is reported. Lower doses of CBD tend to produce anxiolytic-like effect and higher doses produce an anxiogenic behavior (Guimaraes et al., 1990, 1994; Hill and Gorzalka, 2009; Lafenetre et al., 2007). In accordance, in the present study only the lowest dose of CBD increased social interaction in Wistar rats. Considering the absence of an anxiolytic effect of CBD on SHR, several studies have demonstrated that SHRs exhibit decreased basal anxiety-related behaviors when compared to other strains (Calzavara et al., 2004, 2009; Gentsch et al., 1987; Ledoux et al., 1983; Ramos et al., 2002, 2008). In this sense, a floor effect could have prevented the detection of some anxiolytic effect in this strain indicating that this strain would not be suitable to reveal an anxiolytic profile of new compounds.

5. Conclusion

In conclusion, although animal and clinical findings have shown the effectiveness of CBD treatment in schizophrenia, our results suggest that acute CBD does not present an antipsychotic profile for some behaviors of SHR that resembles positive and negative symptoms of schizophrenia. It is noteworthy that we have recently demonstrated a beneficial effect of CBD in reversing the contextual fear conditioning deficit displayed by SHR (similar to antipsychotics — Calzavara et al., 2009), indicating an antipsychotic profile of CBD for emotional processing deficits presented by this strain (Levin et al., 2012). In parallel, our results reinforce the anxiolytic action of CBD (Crippa et al., 2004, 2011). In this scenario, both schizophrenia and anxiety would benefit from novel therapeutic agents, and CBD as well as the endocannabinoid system can play a major role in the advances in novel therapeutic approaches to treat these disorders.

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