Sub-chronic treatment with cannabidiol but not with URB597 induced a mild antidepressant-like effect in diabetic rats

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ABSTRACT

Depression associated with diabetes has been described as a highly debilitating comorbidity. Due to its complex and multifactorial mechanisms, the treatment of depression associated with diabetes represents a clinical challenge. Cannabidiol (CBD), the non-psychotomimetic compound derived from *Cannabis sativa*, has been pointed out as a promising compound for the treatment of several psychiatric disorders. Here, we evaluated the potential antidepressant-like effect of acute or sub-chronic treatment with CBD in diabetic rats using the modified forced swimming test (mFST). Also, to better understand the functionality of the endocannabinoid system in diabetic animals we also evaluated the effect of URB597, a fatty acid amide hydrolase inhibitor. Four weeks after the treatment with streptozotocin (60 mg/kg; i.p.; diabetic group-DBT) or citrate buffer (i.p.; normoglycemic group-NGL), DBT animals received an acute intraperitoneal injection of CBD (0, 0.3, 3, 10, 30 or 60 mg/kg), 1 h before the mFST, or URB597 (0, 0.1, 0.3 or 1 mg/kg) 2 h before the mFST. In another set of experiments, animals were sub-chronically treated with CBD (0, 0.3, 3, 30 or 60 mg/kg i.p.), 24, 5 and 1 h before the mFST or URB597 (0, 0.1, 0.3 or 1 mg/kg i.p.) 24, 5 and 2 h before the mFST. The NGL group was acutely treated with CBD (0, 30 mg/kg i.p.) or URB597 (0, 0.3 mg/kg; i.p.). Acute treatment with either CBD or URB induced an antidepressant-like effect in NGL rats, but not in DBT rats. However, sub-chronic treatment with CBD (only at a dose of 30 mg/kg), but not with URB597, induced a mild antidepressant-like effect in DBT animals. Neither body weight nor blood glucose levels were altered by treatments. Considering the importance of the endocannabinoid system to the mechanism of action of many antidepressant drugs, the mild antidepressant-like effect of the sub-chronic treatment with CBD, but not with URB597 does not invalidate the importance of deepening the studies involving the endocannabinoid system particularly in DBT animals.

1. Introduction

According to the International Diabetes Federation [1], the number of diabetic patients is more than 425 million people worldwide, a number that can reach 642 million in 2040 [1]. It is known that chronic hyperglycemia leads to micro and macrovascular complications, such as nephropathies, retinopathies, heart diseases and also dementia and depression [2–7]. Clinically, the high prevalence of depression in diabetic patients is well known, as well as an increased risk of patients with DM to develop depression [8–13]. Evidence suggests that this comorbidity may be the result of lifestyle changes such as stress, a non-rigid glycemic control, dietary restriction and/or physiological changes due to the diabetic condition itself [2,3,14].

Although antidepressants in combination with hypoglycemic drugs are the treatment of choice for the depression associated with diabetes, this condition is badly controlled by these drugs with only few of the patients achieving true recovery or remission [2, for review see 15]. Moreover, antidepressants should be cautiously prescribed for diabetic patients as some medications may directly influence glycemic control or interact with hypoglycemic drugs [16–18]. Thus, there is a clear demand to explore novel therapeutic compounds for the management of this important clinical condition whose the therapeutic effect appears quickly after the start of treatment, with fewer side effects and/or fewer risks for the patient and with a higher rate of adhesion and

effectiveness.

In view of this, loads of evidence point out to compounds like cannabidiol (CBD), the most abundant non-psychotomimetic compound present in the *Cannabis sativa*. CBD has recently gained prominence as a therapeutic tool for a wide range of disorders, such as anxiety, epileptic seizure, schizophrenia, chronic pain and also depression [19–23]. Interestingly, studies in humans have shown that CBD is well tolerated and does not appear to induce obvious adverse effects, even after prolonged treatment [24]. Of particular interest, preclinical studies conducted in non-diabetic animals show that CBD induces an antidepressant-like effect [20,25] in a similar manner to the classical antidepressant imipramine after acute or prolonged treatment [21,25].

Regarding to diabetes, studies show that CBD may have beneficial effects in relation to the diabetic state *per se*, reducing the incidence of type 1 diabetes in mice and the early pancreatic inflammation in this type of diabetes [26–28]. Using the streptozotocin-induced diabetes animal model, our group recently showed that acute treatment with CBD (at doses of 3 mg/kg; i.p.) exerted a significant reduction of mechanical allodynia, suggesting that CBD may be effective in the treatment of painful diabetic neuropathy (submitted data).

In terms of depression associated with diabetes, to date, there are no reports of the potential antidepressant effect of CBD, which is the main objective of the present study. Thus, it was evaluated the effect of acute or sub-chronic treatment with CBD in depressive-like behaviors in streptozotocin-induced diabetic rats using the modified forced swimming test. Moreover, given the fact that the endocannabinoid effects are tightly regulated by their degradation rate by fatty acid amide hydrolsase (FAAH) and that the endocannabinoid system seems to be unregulated in diabetic animals [7,29], it was also evaluated the effects of URB597, a FAAH inhibitor that elevates the brain anandamide levels in rodents [30,31].

2. Material and methods

2.1. Animals

Adult male Wistar rats (180–220 g), provided by the Federal University of Paraná, were housed in plastic cages (41 × 32 × 16.5 cm) with food and water available *ad libitum* and maintained in a temperature-controlled room (22 + 2 °C) under 12 h/12 h light/dark cycle (lights on at 7:00 a.m.). All the protocols were performed in accordance with the ethical guidelines of Brazilian legislation on animal welfare and previously approved by the Federal University of Parana Institutional Committee for the Ethical Use of Animals (CEUA/BIO-UFPR; authorization #749). All efforts were made to minimize the number and suffering of the animals used.

2.2. Drugs and treatment

The following drugs were used: streptozotocin (STZ; Santa Cruz Biotechnology Inc., USA), sodium citrate (Merck S.A., Brazil), cyclohexylcarbamic acid 3-carbamoylbiphenyl-3-yl ester (URB597; 0.1; 0.3 or 1 mg/kg; i.p.; Sigma Aldrich, USA), cannabidiol (CBD; 0.3, 3, 10, 30 or 60 mg/kg, i.p.; 99.6% pure was kindly supplied by BSPG-Pharm, Sandwich, United Kingdom). STZ was dissolved in citrate buffer (10 mM, pH 4.5). URB597 was dissolved in 2–3 drops of ethanol and diluted as required in a 1% aqueous solution Tween 80. CBD was freshly diluted in 2%Tween 80 and saline [7,20,32,33].

2.3. Diabetes induction

Type-1 experimental diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) freshly dissolved in citrate buffer (10 mM, pH 4.5) in overnight fasten rats. Hyperglycemia was confirmed 72 h after STZ administration by applying a small volume of peripheral blood collected from the tail on test strips impregnated with glucose oxidase (Accu-Check Active™, Roche) and confirmed again at ending of the behavioral tests. Animals with fasting blood glucose levels ≥ 250 mg/dL were considered diabetic and maintained in the experimental groups [7].

2.4. Modified forced swim test

The potential antidepressant-like effect of CBD or URB597 was investigated using the modified forced swim test (mFST) as described initially by Porsolt et al. [34] and modified by Detke et al. [35]. The test was conducted in two sessions. First, in the pre-test session rats were placed individually to swim in a tank (30 cm × 40 cm height, containing 30 cm of water at 22 + 1 °C) for 15 min. Twenty four hours later, animals were submitted to a 5 min session of forced swim (test) and the session was filmed for later analysis. Every 5 s of interval of the test session, the predominant behavior was evaluated: (1) immobility (except the small movements required to float), (2) swimming (movements through the plastic cylinder) and (3) climbing (movement with the front legs in the cylinder wall in an attempt to leave it). After each session (pre-test and test session), the animals were removed and allowed to dry in a separate cage before being returned to their home cages and the tank was cleaned.

2.5. Open-field test

The open field test (OFT) was conducted as described previously [7]. Briefly, all the animals were placed in the center of a wooden rectangular open field (40 × 50 × 63 cm) with a floor divided into 9 rectangular units. The number of squares crossed with all four paws in an interval of 5 min was observed and quantified as a parameter of general motor activity.

2.6. Experimental design

In the first set of experiments, four weeks after the treatment with STZ, diabetic (DBT) animals received an acute injection of CBD (0, 0.3, 3, 10, 30 or 60 mg/kg i.p.). Normoglycemic animals (NGL; treated with citrate buffer, vehicle of STZ) received an acute injection of CBD (at a dose of 30 mg/kg; i.p.) 1 h before the OFT followed by the mFST. Independent groups of DBT animals received an acute injection of URB597 (0, 0.1, 0.3 or 1 mg/kg; i.p.) and NGL animals received an acute injection of URB597 (0.3 mg/kg; i.p.) 2 h before the OFT followed by the mFST. The second set of experiments aimed to evaluate the effect of sub-chronic treatment with CBD or URB597. Thus, DBT animals were submitted to the regimen of 3 intraperitoneal injections of CBD (0, 0.3, 3, 30 or 60 mg/kg), 24, 5 and 1 h before the OFT followed by the mFST or URB597 (0, 0.1, 0.3 or 1 mg/kg) 24, 5 and 2 h before the OFT followed the mFST.

The doses of CBD were based on previous studies of our group in NGL rats [20]. URB597 doses have also been based on prior literature studies using normoglycemic animals [36]. The sub-chronic administration regimen for CBD or URB597 (24 h, 5 h, 1 or 2 h before the forced swimming test, respectively) was proposed by Porsolt et al. [34], when validating this test for the screening of drugs with antidepressant potential. Specifically for the URB597, the time of two hours was chosen for the last treatment since at this time there is a significant elevation of brain endocannabinoids [36]. The choice of only one dose of cannabidiol or URB597 to be tested in normoglycemic animals was based on the fact that the antidepressant potential of these drugs has previously been explored in other studies [20,37,38].

In all set of experiments, animals had their body weight and blood glucose checked weekly.

All behavioral experiments were conducted by an experimenter blind to treatments, but not blind to the diabetic condition since diabetic animals exhibit typical physiological changes such as polyuria, polyphagia, polydipsia and weight gain reduction.
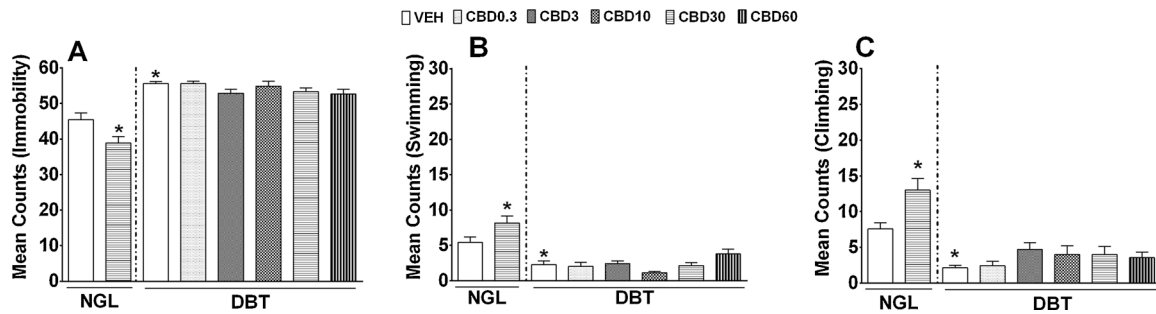
☐VEH ☐CBD0.3 ▨CBD3 ▨CBD10 ☐CBD30 ▥CBD60

Fig. 1. Effect of acute treatment with CBD (0.3, 3, 10, 30 and 60 mg/kg i.p.) or vehicle (VEH) on the frequency of immobility (panel A), swimming (panel B) and climbing (panel C) in NGL and DBT animals submitted to the mFST. Values were expressed as mean ± SEM (n = 7–12). *p < 0.05 when compared with NGL/VEH group.

2.7. Statistical analysis

The Kolmogorov-Smirnov and Levene tests were initially employed to ensure that the data satisfied the criteria for carrying out parametric tests. When criteria were satisfied, the results were reported as the mean + Standard Error of Mean (SEM). The data were analyzed by one-way ANOVA with groups as a single independent factor. When appropriated, Newman–Keuls tests were used for *post hoc* analyses. Differences were considered statistically significant when $p < 0.05$. All the tests were carried out using the GraphPad Prism program (version 6, San Diego, CA, USA).

3. Results

3.1. Acute treatment with CBD did not change the depressive-like behavior in diabetic animals

As showed in Fig. 1 (panels A–C), the one-way ANOVA revealed that the different groups were able to change the frequencies of immobility [$F_{(7,55)} = 20.15$; p < 0.05], swimming [$F_{(7,55)} = 12.69$; p < 0.05] and climbing [$F_{(7,55)} = 11.84$; p < 0.05]. Newman-Keuls *post hoc* test showed that DBT animals presented an increase of the immobility frequency, while the frequencies of swimming and climbing was reduced (p < 0.05) when compared with NGL animals. Interestingly, significant difference was observed between vehicle-treated NGL animals when compared with NGL animals treated with CBD (30 mg/kg; p < 0.05), showing decreased frequency of immobility and increased frequencies of swimming and climbing (p < 0.05). However, the *post hoc* test showed no significant difference between the DBT groups treated with vehicle or CDB (p > 0.05).

3.2. CBD sub-chronic treatment induced antidepressant-like effect in diabetic animals

One-way ANOVA (see Fig. 2, panels A–C) revealed that the different groups were able to change the frequencies of immobility [$F_{(6,72)} = 36.63$; p < 0.05], swimming [$F_{(6,72)} = 9.22$; p < 0.05] and climbing [$F_{(6,72)} = 12.32$; p < 0.05]. Newman-Keuls *post hoc* test showed that DBT animals presented increased frequency of immobility, decreased frequency of swimming and climbing (p < 0.05) when compared with NGL animals. Also, the *post hoc* test showed that CBD treatment (only at the higher doses of 30 mg/kg) significantly decreased the frequency of immobility and increased the frequencies of swimming and climbing (p < 0.05).

3.3. Effect of condition (NGL or DBT) and/or treatment (CBD or vehicle) on glycemia, weight gain and number of crossings evaluated in the open field test

As showed in Table 1, one-way ANOVA revealed that different groups submitted to the CBD acute treatment had altered the glycemia [$F_{(7,55)} = 92,38$; p < 0.05], weight gain [$F_{(7,55)} = 15,92$; p < 0.05] and number of crossings [$F_{(7,55)} = 9,805$; p < 0.05]. The same was observed when a CBD sub-chronic treatment was performed [glycemia: $F_{(6,72)} = 73,56$; p < 0.05; weight gain: $F_{(6,72)} = 17,23$; p < 0.05 and number of crossings: $F_{(6,72)} = 9,114$; p < 0.05]. Newman-Keuls *post hoc* test showed that all DBT animals were different of NGL animals, *i.e.* presented hyperglycemia (p < 0.05), reduced weight gain (p < 0.05) and decrease in the number of crossings in the open field (p < 0.05).

3.4. URB597 acute treatment did not change the depressive-like behavior in diabetic animals

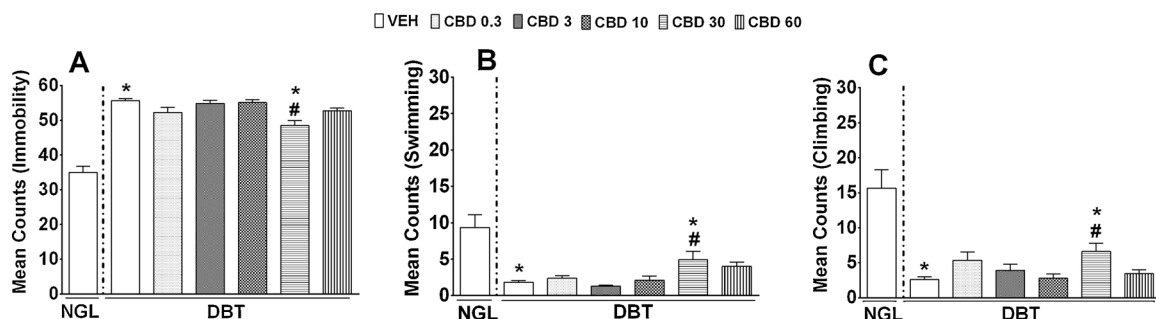As showed in Fig. 3 (panels A–C), the one-way ANOVA revealed that

☐VEH ☐CBD 0.3 ▨CBD 3 ▨CBD 10 ☐CBD 30 ▥CBD 60

Fig. 2. Effect of sub-chronic treatment with CBD (0.3, 3, 10, 30 and 60 mg/kg i.p.) or vehicle (VEH) on the frequency of immobility (panel A), swimming (panel B) and climbing (panel C) response in NGL and DBT animals submitted to mFST. Values were expressed as mean ± SEM (n = 9–13). *p < 0.05 when compared with NGL/VEH group and #p < 0.05 when compared to DBT/VEH group.

Table 1
Effect of condition (NGL or DBT) and/or treatment (CBD or vehicle) on glycemia, weight gain and number of crossings evaluated in the open field test.

Condition/Acute Treatment	Glycemia (mg/dL)	Weight gain (g)	# of crossings
NGL-VEH	102 ± 2.5	140 ± 8	68 ± 1
NGL-CBD (30 mg/kg)	101 ± 1	149 ± 3.5	57 ± 1
DBT-VEH	531 ± 10*	58 ± 3*	38 ± 1*
DBT-CBD (0.3 mg/kg)	510 ± 11.5*	51 ± 3*	35 ± 1.5*
DBT-CBD (3 mg/kg)	520 ± 10*	57 ± 2*	39 ± 1.5*
DBT-CBD (10 mg/kg)	506 ± 11*	55 ± 3*	38 ± 2*
DBT-CBD (30 mg/kg)	506 ± 8*	54 ± 4*	39 ± 1*
DBT-CBD (60 mg/kg)	524 ± 8*	45 ± 2*	30 ± 1*

Condition/Sub-chronic treatment	Glycemia (mg/dL)	Weight gain (g)	# of crossings
NGL-VEH	99 ± 1	121 ± 3	60 ± 2
DBT-VEH	521 ± 5*	48 ± 2*	34 ± 1*
DBT-CBD (0.3 mg/kg)	513 ± 7*	48 ± 2*	35 ± 1*
DBT-CBD (3 mg/kg)	511 ± 6*	45 ± 2*	34 ± 1*
DBT-CBD (10 mg/kg)	501 ± 8*	44 ± 2*	24 ± 1*
DBT-CBD (30 mg/kg)	490 ± 5*	49 ± 1*	40 ± 1*
DBT-CBD (60 mg/kg)	505 ± 6*	44 ± 2*	22 ± 1*

Results are expressed as mean ± SEM; n = 7–14.

  * $p \leq 0.05$ when compared to NGL-VEH.

the different groups were able to change the frequencies of immobility $[F_{(5,47)} = 59.98; p < 0.05]$, swimming $[F_{(5,47)} = 8.16; p < 0.05]$ and climbing $[F_{(5,47)} = 25.91; p < 0.05]$. Newman-Keuls *post hoc* test showed that DBT animals presented increased frequency of immobility, decreased frequency of swimming and climbing ($p < 0.05$) when compared with NGL animals. Interestingly, significant difference was observed between NGL animals treated with vehicle and NGL treated with URB597 (0.3 mg/kg), *i.e.* the treated animals presented decreased frequency of immobility and increased frequencies of swimming and climbing ($p < 0.05$). However, the *post hoc* test showed no significant difference between the DBT groups treated with vehicle or URB597 ($p > 0.05$).

3.5. URB597 sub-chronic treatment did not change the depressive-like behavior in diabetic animals

One-way ANOVA (Fig. 4, panels A–C) revealed that the different groups were able to change the frequency of immobility $[F_{4,42)} = 10.25; p < 0.05]$, swimming $[F_{4,42)} = 5.64; p < 0.05]$ and climbing $[F_{4,42)} = 7.21; p < 0.05]$. Newman-Keuls *post hoc* test showed that DBT animals presented increased frequency of immobility, decreased frequencies of swimming and climbing ($p < 0.05$) when compared with NGL animals. However, the *post hoc* test showed no significant difference between the DBT groups treated with vehicle or URB597 ($p > 0.05$).

3.6. Effect of condition (NGL or DBT) and/or treatment (URB597 or VEH) on glycemia, weight gain and number of crossings evaluated in the open field test

As showed in Table 2, one-way ANOVA revealed that different groups submitted to the URB597 acute treatment had altered the glycemia $[F_{(5,47)} = 271,2; p < 0.05]$, weight gain $[F_{(5,47)} = 25,56; p < 0.05]$ and number of crossings $[F_{(5,47)} = 10,27; p < 0.05]$. The same was observed when a sub-chronic treatment with URB597 was performed [glycemia: $F_{(4,42)} = 49,93; p < 0.05$; weight gain: $F_{(4,42)} = 17,98; p < 0.05$ and number of crossings: $F_{(4,42)} = 4,695; p < 0.05$]. Newman-Keuls *post hoc* test showed that all DBT animals were different of NGL animals, *i.e.* presented hyperglycemia ($p < 0.05$), reduced weight gain ($p < 0.05$) and decrease in the number of crossings in the open field ($p < 0.05$).

4. Discussion

The main finding of this study is that, although both CBD and URB597 induced an antidepressant-like effect when administered acutely to normoglycemic (NGL) animals, these treatments were not able to induce this effect in diabetic (DBT) animals. Interestingly, even after sub-chronic treatment, URB597 did not induce an antidepressant-like effect in DBT animals. Conversely, this regimen of treatment with CBD induced a mild antidepressant-like effect in these rats. This work is, to our knowledge, the first to suggest that CBD has a favorable profile in a similar model predictive of antidepressant-like activity (3 injections [34];) in DBT animals.

As clinically observed, DBT animals exhibit a more pronounced depressive-like behavior when compared to NGL ones [7,17,39–41] evidenced in this study by an increase in the immobility frequency and a reduction in the frequencies of climbing and swimming in the mFST [7,41]. As well demonstrated in the literature, these behaviors reflect dysregulations on the noradrenergic and serotonergic systems [7,39,41,42], which is strictly related to the neurobiology of depression [2,3,43–46].

Even if the behaviors exhibited by NGL animals in the mFST are analyzed separately, they are consistent with depressive-like behaviors, and therefore susceptible to modulation by drugs with antidepressant potential. In this study, the acute treatment with CBD (at a dose of 30 mg/kg) induces an antidepressant-like effect in NGL animals, supported by the reduction of the frequency of immobility and an increase of swimming and climbing frequencies (Fig. 1). This acute CBD antidepressant-like propriety has already been observed in both rats and mice [20,25]. Although it was not the aim of this study, the mechanisms by which the CBD exhibits this effect are multiple and complex and therefore, not fully understood. In this sense, it has been proposed that CBD may exert the antidepressant-like effect acting directly or indirectly on cannabinoid receptors CB1 and/or CB2 and also, although controversial, through a low affinity antagonistic action on both cannabinoid receptors [24,47–49]. CBD can also exert the antidepressant-like effect by increasing the endocannabinoid tone through the
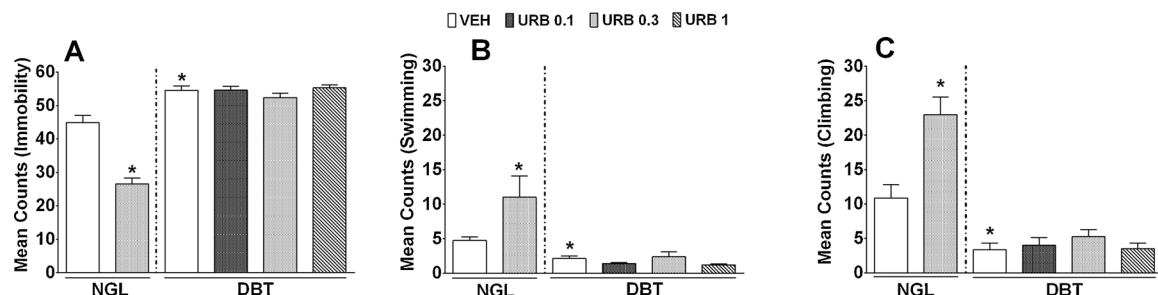

Fig. 3. Effect of acute treatment with URB597 (0.1, 0.3, 1 mg/kg i.p.) or vehicle (VEH) on the frequency of immobility (panel A), swimming (panel B) and climbing (panel C) in NGL and DBT animals submitted to the mFST. Values were expressed as mean ± SEM (n = 8–10). * $p < 0.05$ when compared with NGL/VEH group.
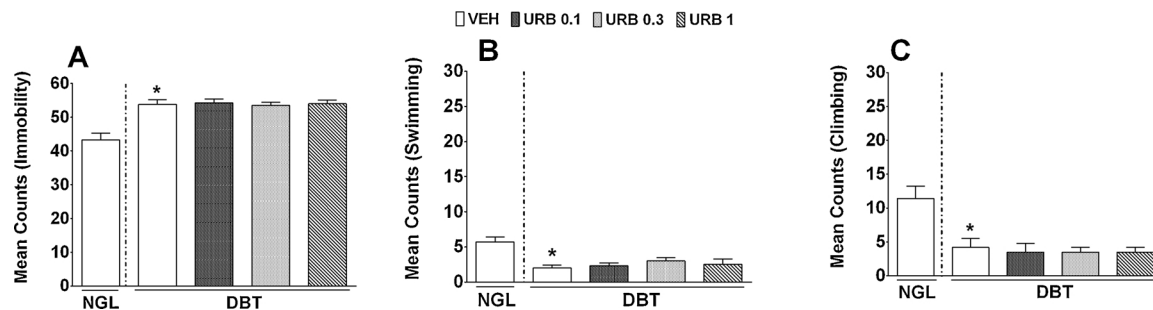
VEH ■URB 0.1 □URB 0.3 ▨URB 1

Fig. 4. Effect of sub-chronic treatment with URB597 (0.1, 0.3, 1 mg/kg i.p.) or vehicle (VEH) on the frequency of immobility (panel A), swimming (panel B) and climbing (panel C) in NGL and DBT animals submitted to the mFST. Values were expressed as mean ± SEM (n = 7–10). *p < 0.05 when compared with NGL/VEH group.

Table 2
Effect of condition (NGL or DBT) and/or treatment (URB597 or VEH) on glycemia, weight gain and number of crossings evaluated in the open field test.

Condition/Acute Treatment	Glycemia (mg/dL)	Weight gain (g)	# of crossings
NGL-VEH	104 ± 1.5	116 ± 3	50 ± 1
NGL-URB597 (0.3 mg/kg)	106 ± 1	119 ± 2.5	53 ± 1
DBT-VEH	535 ± 7*	50 ± 3*	37 ± 1*
DBT-URB597 (0.1 mg/kg)	543 ± 5*	49 ± 2*	31 ± 1*
DBT-URB597 (0.3 mg/kg)	550 ± 5*	55 ± 2*	38 ± 1*
DBT-URB597 (1 mg/kg)	546 ± 5*	52 ± 1*	31 ± 1*

Condition/Subchronic treatment	Glycemia (mg/dL)	Weight gain (g)	# of crossings
NGL-VEH	105 ± 1	117 ± 3	63 ± 2
DBT-VEH	471 ± 7*	49 ± 2*	41 ± 1*
DBT-URB597 (0.1 mg/kg)	459 ± 8*	46 ± 2*	42 ± 1*
DBT-URB597 (0.3 mg/kg)	498 ± 6*	49 ± 2*	40 ± 1*
DBT-URB597 (1 mg/kg)	478 ± 7*	48 ± 2*	42 ± 1*

Results are expressed as mean ± SEM; n = 7–10.
 * p ≤ 0.05 when compared to NGL-VEH.

inhibition of fatty acid amide hydrolase (FAAH), leading to anandamide increase in the central nervous system [50]. Although there are these hypotheses, it seems to be the serotoninergic system most related to many biological effects of CBD. In fact, the antidepressant-like effect of CBD seems to be dependent on 5-HT1 A serotonergic receptors [25] and also due to increased levels of serotonin and glutamate in the prefrontal cortical area [33]. Interestingly, both antidepressant-like effect and enhanced cortical 5-HT/glutamate neurotransmission induced by CBD were prevented by blocking 5-HT1 A receptor, reinforcing the involvement of this serotonergic receptor.

In contrast, in DBT animals the acute treatment with CBD (30 or even a higher dose of 60 mg/kg) was not able to induce an antidepressant-like effect. Many hypotheses could be relevant to explain this lack of acute CBD effect. The DBT state is closely related to hyperglycemia-induced oxidative stress [7,40,51,52] decreased hippocampal cell proliferation [53], neuroinflammation [41,54], dysregulation of the hypothalamus-pituitary-adrenal-axis [55,56], and also dysregulation of endocannabinoid system [7,29,57], which may account for the development of depression. Interestingly, DBT animals also showed an increase in the activity of the monoamine oxidase enzyme and consequently an elevation in the 5-HT metabolism, leading to a low level of 5-HT in brain areas [39,41,44,58–60]. Considering the serotonergic system as a multifactorial target in the pathophysiology of depression associated with diabetes, a single CBD injection may not be sufficient to counterbalance the chronic consequences of the DBT state. Nonetheless, the sub-chronic treatment with CBD (only at a dose of 30 mg/kg) was able to induce an antidepressant-like effect in DBT animals. The effective sub-chronic dose of CBD (30 mg/kg i.p.) was able to reduce the frequency of immobility and increase the frequency of

swimming and climbing in DBT animals (Fig. 2), suggesting an improvement in the monoaminergic function. Curiously, the sub-chronic treatment with CBD at the highest dose (60 mg/kg) was not able to reverse the depressive-like behavior in DBT animals, in a typical U-curve pattern of cannabinoid-mediated effects.

The antidepressant-like effect of CBD does not seem to depend on the improvement of general health status or hyperglycemia since the sub-chronic treatment with CBD did not change these parameters (Table 1). This absence of effect may be due to the short time of treatment with the CBD. In that sense, it has been noticed that a more prolonged treatment with CBD has a protective effect on pancreatic cells, reducing the markers of inflammation in the microcirculation of pancreas from non-obese DBT mice [27] and the insulitis [26]. In addition, as already shown in previous studies, DBT animals present a reduction in locomotor activity (Tables 1 and 2 [7,61,62];) that was not altered by acute or sub-chronic treatment with CBD. This reduction in locomotor activity seems not to be interfering in the antidepressant-like effect of sub-chronic injection of CBD since this regimen of treatment induced not only a reduction of immobility frequency but also a significant increase on the swimming and climbing frequencies during the mFST.

Another set of experiments was designed to further explore the role of the endocannabinoid system in the maintenance of depressive-like status in DBT animals, since a dysregulation in this system can be related to a more pronounced depressive-like behavior in DBT animals, as observed previously in our lab [7]. Therefore, the effects of acute or sub-chronic treatment with the FAAH inhibitor URB597 were tested. Our data showed that both acute and sub-chronic treatment with URB597 (0.1, 0.3 or 1 mg/kg; i.p.) did not change the frequencies of immobility, climbing and swimming of DBT animals submitted to mFST (Figs. 3 and 4). These doses and regimen of treatments were not able to influence the reduced weight gain, the hyperglycemia and the reduced locomotor activity of the DBT animals (Table 2). Nevertheless, the acute treatment with URB597 (0.3 mg/kg, i.p.) showed an antidepressant-like effect in NGL animals submitted to mFST, evidenced by a significant decrease in the frequency of immobility and an increase in the frequencies of climbing and swimming (Fig. 3), as already observed previously [37,38].

It is known that the URB597 increases the anandamide levels in the central nervous system, leading to an antidepressant-like effect [36,37,63], which is related to the modulation of serotonin and norepinephrine neurotransmission through CB1 receptor activation [37,64–66]. Corroborating the importance of the serotonergic system in the modulation of the depression and therefore in the effect of drugs with antidepressant profile, Bambico et al. [66] observed that para-chlorophenylalanine, a serotonin synthesis inhibitor, prevented the URB597-mediated antidepressant-like effect evaluated in the FST. Moreover, a single URB597 administration was able to gradually increase the serotonin neuron-firing rate in dorsal raphe nucleus, consistent with other previous findings [37,64,65]. Then, the absence of

antidepressant-like effect after acute or sub-chronic treatment with URB597 in DBT animals (Figs. 3 and 4) can be due to a dysregulation of both serotonergic and endocannabinoid systems observed in these animals [39,41,44,58–60]. Perhaps the reestablishment of neurotransmission is only achieved after an even longer treatment in DBT animals. In this sense, it has been demonstrated that only after a chronic treatment with URB597 rats exposed to moderate chronic stress exhibited a reduction of the depressive-like behaviors, an increase in the anandamide levels in some brain areas and also a normalization of the body weight gain [36].

Considering the importance of raising the levels of endocannabinoids in some encephalic areas related to the emotions to the mechanism of action of many antidepressant drugs (as evidenced by Smaga et al. [32]), it is plausible to hypothesize that the endocannabinoid system may serve as a target for drug design and discovery of new therapies for depression, especially depression associated with diabetes. Even after the observation of the mild antidepressant-like effect of the sub-chronic treatment with CBD, but not with URB597, the data obtained in this study do not invalidate the importance of deepening the studies involving the endocannabinoid system particularly in DBT animals. A better understanding of the pathophysiological mechanisms that associate diabetes with depression will permit the proposition of more effective drugs, with fewer side effects and faster clinical response.

Contributors

Janaína M Zanoveli and Joice Maria da Cunha proposed the study. Helen de Morais, Yane C. Chaves, Ana Paula F Waltrick and Carlos H.A. Jesus conducted the behavioral tests. José A Crippa and Karina Genaro were responsible for the donation of cannabidiol. All of the authors contributed to the data analysis and interpretation, wrote the manuscript and approved the final article.

Conflict of interest statement

JAC is co-inventor (Mechoulam R, JC, Guimaraes FS, AZ, JH, Breuer A) of the patent "Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023" Def. US no. Reg. 62193296; 29/07/2015; INPI on 19/08/2015 (BR1120150164927). The University of São Paulo has licensed the patent to Phytecs Pharm (USP Resolution No. 15.1.130002.1.1). The University of São Paulo has an agreement with Prati-Donaduzzi (Toledo, Brazil) to "develop a pharmaceutical product containing synthetic cannabidiol and prove its safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson's disease, and anxiety disorders." JAC received travel support from and is medical advisor of BSPG-Pharm.

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