Behavioural Brain Research 351 (2018) 1–3

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Acute oral cannabidiolic acid methyl ester reduces depression-like behavior in two genetic animal models of depression

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ARTICLE INFO

Keywords:
Major depression disorder (MDD)
Cannabidiolic acid methyl ester
Genetic animal models of depression
Wistar–Kyoto (WKY)
Flinders Sensitive Line (FSL)
Forced swim test (FST)

ABSTRACT

Background and purpose: Cannabidiolic acid methyl ester (HU-580) was recently shown to reduce stress-induced anxiety-like behavior in rats. The aim of this study was to examine the antidepressant effect of HU-580 in two different rat models of depression.

Experimental approach: Using the forced swim test (FST), we evaluated the effect of HU-580 in 43 Wistar–Kyoto (WKY) and 23 Flinders Sensitive Line (FSL) adult male rats.

Key results: 1 mg/kg HU-580 reduced immobility and increased swimming in WKY rats, compared to vehicle-treated controls ($p < 0.05$). This dose exerted similar effects in FSL rats ($p < 0.05$).

Conclusion and implications: This is the first report of antidepressant efficacy of HU-580. These findings expand the very limited existent results, suggesting that HU-580 is a potent anxiolytic agent. Taken together with its chemical stability, HU-580 emerges as a candidate for a future antidepressant medication.

Major depressive disorder (MDD) is a public health concern with a lifetime prevalence of 10%–15% in the general population [1]. In many cases, antidepressant drugs ease the clinical symptoms, yet these medications do not affect a substantial portion of the patients or their remission may be partial with side effects. There is increasing evidence to support a role for the endocannabinoid system (ECB system) in the neurobiology of depression [2]. The ECB system can exert its influence through its regulation of hypothalamic–pituitary–adrenal (HPA) axis activity, since changes in the HPA axis are characteristic of major depression. Indeed, typical antidepressant agents normalize the hyperactivity of the HPA axis and they facilitate endocannabinoid neurotransmission through uptake inhibitors or metabolic enzyme inhibitors suggesting that the ECB system may play a role in both the pathophysiology and treatment of MDD [3,4].

Cannabidiol (CBD) is the major non-psychotogenic phytocannabinoid compound present in the plant Cannabis sativa, making up to 40% of Cannabis extracts [5]. It was repeatedly shown to induce anxiolytic activity in preclinical and clinical studies [6,7]. Antidepressant effects have also been reported [8,9]. Recently, a study from our laboratory, in an animal model, indicated that oral 30 mg/kg CBD had a pro-hedonic effect in the saccharin preference test (SPT [10]) and antidepressant effects in the forced swim test (Shbiro et al., submitted).

In contrast to the extensive knowledge on CBD, there is very limited literature on cannabidiolic acid (CBDA), also a major constituent of the C. sativa plant, which may be due to its instability. The cannabinoid acids are precursors of the natural cannabinoids [11] potentially lowering the amount of drug required to induce effects. In one study, CBDA increased the effect of a 5-HT1A receptor agonist and increased the activity of this receptor, suppressing nausea [12]. The effect on the 5-HT1A receptor is highly relevant to pathophysiology and treatment of human depression and anxiety [13]. Another study indicated that CBDA suppressed acute nausea produced by LiCl in rats [11]. The effective doses of CBDA, which weakened the conditioned response of gasp nausea, were 1000 times lower than the effective doses of CBD (between 1–5 mg/kg; [12,14].

Unfortunately, CBDA decarboxylates into CBD [15]. This process is

Abbreviations: HU-580, cannabidiolic acid methyl ester; FST, forced swim test; WKY, Wistar–Kyoto; FSL, Flinders Sensitive Line; ECB system, the endocannabinoid system; CBDA, cannabidiolic acid

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https://doi.org/10.1016/j.bbr.2018.05.027
Received 30 March 2018; Received in revised form 15 May 2018; Accepted 25 May 2018
Available online 31 May 2018

enhanced by heat, indicating a relative instability of CBDA, thus lowering its potential to be a future medication [16]. However, its stable analogue, CBDA methyl ester (HU-580) has been recently shown to be more potent than CBDA and to possess anxiolytic properties in a stress-induced anxiety-like behavior Sprague-Dawley rat model [17].

To expand this preliminary knowledge on HU-580, in the present study we examined for the first time its antidepressant effect in two different rat models for depression. This was explored using the forced swim test (FST) after acute oral ingestion of different doses of HU-580 in male rats of the Wistar- Kyoto (WKY) genetic model of depression and one dose in the Flinders Sensitive Line (FSL) genetic model. Both WKY and the FSL rat models have many behavioral and physiological endophenotypes that are often present in MDD, making them valuable models for studying depression (for reviews see [18,19]). While the WKY model exhibits both depressive-like and anxiety-like behavior, the FSL model typically does not exhibit anxiety-like behavior [18,19]. The depressive-like behavior of FSL rats is mostly exhibited as increased immobility on the FST, while WKY rats also demonstrate in addition to this phenotype, an anhedonia-like profile [18,19].

The study included forty-three WKY (mean weight 205 g) and twenty-three FSL (mean weight 308 g) adult male rats approximately 70-days-old. The rats were provided by Bar-Ilan University's colony, progenitors were provided by Prof. Overstreet (FSL) or purchased from Envigo, Inc. Rats were housed in polycarbonate cages (38 × 21 × 18 cm) with standard bedding, 2 per cage, in a temperature controlled facility (22 + 1 °C), under 12 h-12 h light:dark cycle (lights on at 07:00). Food and water were available *ad libitum* and each cage contained a segment of wide plastic drainage pipe for enrichment. The study protocol adheres to the ARRIVE guidelines, was in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and was approved by the Institutional Animal Care and Use Committee.

In on our previous study (Shbiro et al., submitted), we assessed the effect of acute orally consumed CBD in the same strains and sex as in the current study, using the same (FST) paradigm. In that study, on the immobility measure, we found an effect size of 1.7 ($p < 0.05$, with a power of 99). Based on these data, power analysis showed that the minimal number of subjects per group needed to find this effect size, with power = 90, is 9. Therefore in the current study we aimed for a N = 12 per group. This can allow for potential subject attrition and/or rejection of outliers.

CBDA methyl ester (HU-580) was synthesized as previously described [20]. The forced swim test (FST) was carried out similar to the one described by Porsolt et al. [21] with minor modifications. A Plexiglas cylinder, 45.5 cm tall, 20 cm diameter was filled to 30 cm with water at 24 ± 0.5 °C. The animals were immersed in the Plexiglas cylinder for 5 min.

The following measurements were recorded: duration of immobility, duration of struggling behaviors (measured online using a stopwatch) and number of dives. The criterion for immobility was making only the minimal movements necessary to keep the head above water, with no forelimb movement. The criterion for struggling was making active forepaw movements in and out of the water including climbing behavior. At completion of the test, animals were dried off with a towel. The cylinder was cleaned and water changed between test animals.

In the first study, male WKY rats received either 0.1, 1 or 5 mg/kg of HU-580 dissolved in 70 µl ethanol or 70 µl of ethanol (vehicle) that was laced onto a pellet of high fat rodent diet (D12492 Research Diets, Inc. Rodent diet with 60% Fat, NJ USA). The animals consumed the pellet without any need of coercion. Behavioral testing began 2 h after the pellet was consumed (the same time frame in which we found antidepressive-like effects of CBD (Shoval et al., 2016; Shbiro et al., submitted). Since only one dose of the drug was found effective, in the second study, the same procedure was conducted with male FSL rats using the dose of 1 mg/kg HU-580 compared to vehicle control. Rats were allocated to the treatment groups in a random manner with a limitation: since they were housed 2 per cage, in any given cage one was heavier (and potentially also dominant) than the other. When electing rats for drug treatment the relative weight (heavier/lighter) per cage was counter-balanced within each treatment group.

Two researchers performed the experiments. One (DS) prepared the drug solutions, and was therefore not "blind" to the group assignment of the rats. The other (SA) was totally "blind". Both researchers observed all rats together, switching roles in scoring Floating or Struggling between animals randomly.

One-way multivariate analysis of variance (MANOVA) was followed by one-way ANOVAs on each dependent measure. For the 4-group WKY study, post-hoc one-tailed Dunnett's test was used for comparisons between each dose and the control, vehicle group. MANOVAs included immobility and swimming as the dependent variables. Struggling was not included in the MANOVAs to allow for degrees of freedom in the analysis.

Towards the end of the WKY experiment we ran out of HU-580. Therefore, only 10, out of 12 planned rats in the 1 mg/kg group were tested. At the data analysis stage, data from 3 rats were excluded because they were identified as outliers by SPSS in the boxplot procedure. Thus, for data analysis we had N = 12 in the vehicle and 5 mg/kg groups, N = 11 in the 0.1 mg/kg group, and N = 8 in the 1 mg/kg group.

WKY: One way MANOVA performed on the variables: immobility and swimming revealed a significant effect of drug ($F(6,76) = 2.924$, $p < 0.05$). One way ANOVAs showed significant effects of drug on immobility ($F(3,39) = 5.194$, $p < 0.05$) and swimming ($F(3,39) = 5.648$, $p < 0.05$). Post-hoc Dunnett's tests further revealed that WKY rats treated with 1 mg/kg HU-580 were significantly less immobile ($p < 0.05$) and swam significantly more ($p < 0.05$) than WKY rats treated with vehicle (Fig. 1).

At the data analysis stage, in the FSL study, data from 1 rat was excluded because it was identified as an outlier by SPSS in the boxplot procedure. Thus, for data analysis we had N = 11 in the vehicle group and N = 11 in the 1 mg/kg group.

FSL: One way MANOVA performed on the variables: immobility and swimming, revealed a significant effect of HU-580 ($F(1,21) = 7.375$, $p < 0.01$). HU-580 significantly reduced immobility and increased swimming ($p < 0.05$) (Fig. 2).

In the present study we demonstrated that HU-580 reduced depression-like behavior in two different genetic animal models of depression. This is the first report of the antidepressant efficacy of HU-580. These findings are consistent with the only existing paper in the literature, which suggested that HU-580 is a potent anxiolytic agent [17]. Whereas, a stress-induced anxiety-like behavior Sprague-Dawley rat model was used in the previous study [17], we expanded the

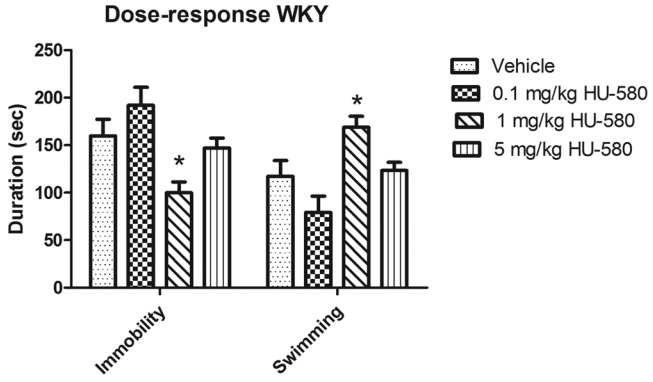

Fig. 1. Duration of immobility and swimming (mean + SEM) of male Wistar Kyoto (WKY) rats. Rats received either vehicle (n = 12) or 0.1 (n = 11), 1 (n = 8), or 5 (n = 12) mg/kg HU-580 orally ingested 2 h before the FST. * $p < .05$.

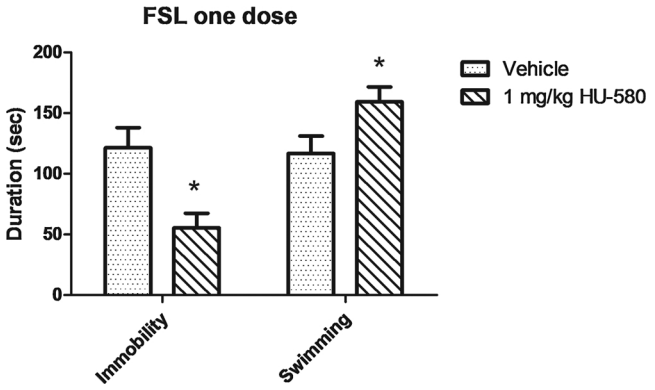

Fig. 2. Duration of immobility and swimming (mean + SEM) of male Flinders Sensitive Line (FSL) rats. Rats received either vehicle (n = 11) or 1 mg/kg HU-580 (n = 12). * p < .05.

knowledge to two different genetic models for depression.

The significance of the findings obtained in this study is the functional antidepressant similarity between HU-580 and CBD, with apparent higher potency of HU-580, based on our previous results with CBD administered under the same conditions ([10]; Shbiro et al., submitted). Taken together with its chemical stability, HU-580 emerges as a candidate for a future antidepressant medication. The results indicate a biphasic effect (higher effect at 1 mg/kg than at 5 mg/kg). This is frequently seen with cannabinoids [8–10].

Compared with the higher dosages of CBD used in previous reports to ameliorate depression-like behavior (200 mg/kg [8], 30 mg/kg [9], FST in mice [8]; 30 mg/kg, SPT and FSL in rats [10 & Shbiro et al., submitted]), a dose as low as 1 mg/kg was shown in the present study to be effective in both the WKY and the FSL models. In line with the previous report on an anxiolytic effect [17], these findings reaffirm the potency of HU-580, which is a pivotal demand of a novel compound to be developed into a medication, as lower effective dosages may cause less adverse effects.

Finding similar results in the two different models of depression strengthens the convergent validity of our findings, but this was not necessarily expected. On the one hand, we recently found effects of acute administration of CBD in FSL rats (Shbiro et al., submitted). However, the FSL model is known to be resistant to acute administration of antidepressants, which typically need to be administered for at least 5 days, and often 2 weeks, before becoming affective in this strain [19].

Limitations: The findings of this study should be interpreted in light of its limitations. First, only male rats were tested, as the amount of drug available was limited and we chose to start investigating this topic in the more-studied sex. Depression is twice as common among women compared with men [22], yet females remain underrepresented in preclinical research [23]. Thus, there is need to replicate the results of the current study with female rats of both genetic models of depression. Second, another core symptom of depression is anhedonia (DSM-V [24]). Hence, further research is needed to explore whether CBDA-ME has pro- hedonic effects as well, as we have shown for CBD [10]. Third, in the present study we used only one paradigm, the forced swim test, to evaluate the antidepressant effect of HU-580. Future studies should replicate this effect using other well-established relevant behavioral tests, as well as further explore the anxiolytic properties using other behavioral tests.

In conclusion, the results of these experiments provide support for a potent anti-depression-like effect of acute oral ingestion of a low dose (1 mg/kg) of the methyl ester form of CBDA, HU-580 in two rat models. Future studies will shed more light on its efficacy in animal models and human studies.

Competing interests

None.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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