Physiology & Behavior 135 (2014) 119–124

Contents lists available at ScienceDirect

Physiology & Behavior

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

β-Caryophyllene, a CB_2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice

Amine Bahi [a,*,1], Shamma Al Mansouri [a], Elyazia Al Memari [a], Mouza Al Ameri [a], Syed M Nurulain [b], Shreesh Ojha [b,1]

[a] Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates
[b] Department of Pharmacology & Therapeutics, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

HIGHLIGHTS

- β-Caryophyllene (BCP) was tested in anxiety and depression-like models.
- BCP displayed anti-anxiety like effects in C57BL/6 mice.
- BCP was anti-depressant in C57BL/6 mice.
- BCP effects in depression and anxiety were abrogated by CB_2 antagonist AM630.
- CB_2 receptors may be targeted in the treatment of anxiety and depression.

ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form 29 May 2014
Accepted 4 June 2014
Available online 13 June 2014

Keywords:
AM630
Anxiety
β-Caryophyllene
CB_2 receptors
Depression

ABSTRACT

Recent evidence suggests that the cannabinoid receptor subtype 2 (CB_2) is implicated in anxiety and depression disorders, although few systematic studies in laboratory animals have been reported. The aim of the current experiments was to test the effects of the CB_2 receptor potent-selective agonist β-caryophyllene (BCP) in animals subjected to models of anxiolytic- and antidepressant-like effects. Therefore effects of BCP (50 mg/kg) on anxiety were assessed using the elevated plus maze (EPM), open field (OF), and marble burying test (MBT). However for depression, the novelty-suppressed feeding (NSF), tail suspension test (TST), and forced swim tests (FST) were used. Results indicated that adult mice receiving BCP showed amelioration of all the parameters observed in the EPM test. Also, BCP significantly increased the time spent in the center of the arena without altering the general motor activity in the OF test. This dose was also able to decrease the number of buried marbles and time spent digging in the MBT, suggesting an anti-compulsive-like effect. In addition, the systemic administration of BCP reduced immobility time in the TST and the FST. Finally, BCP treatment decreased feeding latency in the NSF test. Most importantly, pre-administration of the CB_2 receptor antagonist AM630, fully abrogated the anxiolytic and the anti-depressant effects of BCP. Taken together, these preclinical results suggest that CB_2 receptors may provide alternative therapeutic targets for the treatment of anxiety and depression. The possibility that BCP may ameliorate the symptoms of these mood disorders offers exciting prospects for future studies.

1. Introduction

Depression and anxiety disorders are considered as the most common psychiatric disorders which often exist together rather than as separate syndromes [1]. Their incidence is rising worldwide and affecting millions of individuals. The impact on productivity and quality of life is significant because the disorders affect more than 20% of the adult population at some time during their life mostly in the productive age periods. During recent decades, the overall risk of suffering from depression has increased and the age of onset has decreased.

Recently, a growing body of evidence from pharmacological and genetic studies has suggested that the endocannabinoid system (ECS) is involved in the regulation of mood [2–4] and anxiety disorders [5,6]. The endocannabinoids act through cannabinoid receptors, CB_1 and CB_2, which couple to the $G\alpha_{i/o}$ class of G-proteins and have presynaptic or postsynaptic distribution in the brain [7–9]. Since the discovery of the ECS numerous investigations addressed the role of CB_1 receptors in mediating all CNS effects such as anxiety and depression. The

Abbreviations: BCP, β-caryophyllene; EPM, elevated plus maze; FST, forced swim test; MBT, marble burying test; NSF, novelty suppressed feeding; OF, open field; TST, tail suspension test.

* Corresponding author at: Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain, United Arab Emirates. Tel.: +971 3 7137 516; fax +971 3 7672 033.
E-mail address: amine.bahi@uaeu.ac.ae (A. Bahi).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.physbeh.2014.06.003
0031-9384/

appearance of anxiety and depression-like behavior as adverse events with CB_1 receptor antagonists or the increased CB_1 receptor activity or mutation has been shown in preclinical and clinical studies. The effects of cannabinoid drugs mediated by CB_1 receptors on mood and anxiety are biphasic, anxiolytic with low doses and anxiogenic with high doses [10]. As the CB_2 receptors were considered to be absent in the brain, the original assumption was that the CB_1 receptors were responsible for the anxiety and mood disorders.

The CB_2 receptor which was thought to be restricted to immune cells and peripheral tissues, has been well identified in the brain [11] and pointed out the involvement of CB_2 receptors in anxiety and depressive-related disorders [for review see: [12–15]]. Recently emerging studies are suggesting that drugs acting thru CB_2 receptors could be exploited as novel pharmacological agents in the treatment of depression and anxiety. Also, agents targeting CB_2 receptors have garnered attention as they are devoid of CB_1-mediated psychotropic adverse effects. Additionally the CB_2 receptor modulators hold numerous beneficial pharmacological effects over existing benzodiazepines (BZDs) and selective serotonin reuptake inhibitor (SSRI) drugs which are considered as mainstay of treatment in anxiety and depressive disorders, despite of serious side effects such as sedation, ataxia, amnesia and dependence [for review see: [16,17]].

Considering the need for novel compounds that could improve conventional therapies, a large number of novel synthetic [18,19] and natural [20,21] CB_2 receptor ligands have also been intensively investigated. Among them β-caryophyllene (BCP) a naturally available sesquiterpene is pharmacologically a selective agonist for CB_2 receptors. It represents a dietary phytocannabinoid and the United States Food and Drug Administration (USFDA) has approved it as food additive (approval reference no. 21CFR172.515). It's widely found in high concentrations in many plants and spices such as oregano, cinnamon, clove, rosemary, thyme, and black pepper [22–24]. Interestingly, it has shown therapeutic potential in ulcerative colitis, neuropathic pain, endometriosis, renal protection and anxiety [25–29]. Very recently, Galdino and co-workers investigated the anxiolytic activity of BCP and the possible mechanisms of action specifically by evaluating the role of $GABA_A$/BZD or 5-HT_{1A} receptors via pretreatment with flumazenil, a $GABA_A$ receptor antagonist and NAN-190, a selective 5-HT_{1A} receptor antagonist. Their results have shown that neither of the antagonists was able to antagonize the anxiolytic effects and suggested that non-BZD/5-HT_{1A} receptors are involved in the anxiolytic effects [30]. Recently, BCP has been shown to confer protection against ulcerative colitis and nephrotoxicity in a CB_2 receptor-dependent manner [31,32].

Accordingly, in the present study the anxiolytic and antidepressant effects of BCP in mouse models of anxiety- and depression-like behaviors were investigated. More importantly and in order to elucidate the CB_2 receptor-mediated mechanism in the anxiolytic- and antidepressant-actions of BCP, the mice were administered the CB_2 receptor selective antagonist AM630 [31] prior to the BCP treatment.

2. Materials and methods

2.1. Animals

The experiments were performed in adult male C57BL/6 mice weighing 21–26 g obtained from the local experimental animal breeding facility of the College of Medicine & Health Sciences (CMHS), United Arab Emirates University and maintained in a temperature-controlled environment (~22 °C). Mice were housed in groups of 5/cage in a 12 h light/dark cycle (lights on at 6 am), with ad libitum access to food and water. Standard rodent chow diet was obtained from the National Feed and Flour Production and Marketing Company LLC (Abu Dhabi, UAE). Procedures were approved by the local Ethical Committee (protocol number: A25-13).

2.2. Drugs

The CB_2 receptor agonist, β-caryophyllene (BCP; 50 mg/kg) was diluted in olive oil. However, the CB_2 receptor antagonist [(6-iodopravadoline or 6-iodo-2-methyl-1-(2-morpholinoethyl)-1H-indol-3-yl)(4-methoxyphenyl) methanone], AM630 (3 mg/kg) was diluted in 2.5% DMSO. Both, the drugs and solvents were obtained from Sigma-Aldrich (MO, USA) and injected intraperitoneally (i.p.) with a volume of 10 mL/kg adjusted to body weight. The time between the two injections was 15 min and behavioral testing was performed 15 min after the 2nd injection. This pretreatment–treatment combination created three test groups: DMSO-Oil ($n = 7$), DMSO-BCP ($n = 10$), AM630-BCP ($n = 8$).

2.3. Behavioral experiments: apparatus and procedures

2.3.1. Elevated plus maze (EPM) test

The anxiety-like behavior was assessed using a wooden EPM apparatus as described previously [33,34]. Briefly, the maze consisted of two opposite open-arms, 40×6 cm^2 and two enclosed arms, $40 \times 6 \times 20$ cm^3, with a 6×6 cm^2 central area and elevated 40 cm above the floor. During each 5 min EPM session, the amount of time spent with head and forepaws on the open or closed arm of the maze as well as the number of entries into each arm were manually scored by a trained observer. It is well established that laboratory rodents naturally avoid the open arms of the EPM and anxiolytic compounds typically increase the exploration of these arms without changing the number of enclosed-arm entries, which is an index of locomotor activity [35]. The maze was thoroughly cleaned with 70% ethanol between tests.

2.3.2. Open field (OF) test

In this test, the animals were placed in the center of a square open field (OF) arena 32×32 cm^2 surrounded by a 20 cm high Plexiglas wall in which the exploratory activity was recorded during 5 min as described previously [33]. The floor was divided into 64 equal grids by black lines. We designated the central sixteen grids along and the rest grids as “center area” and “peripheral area”, respectively. The animal was placed in the center of the open field at the beginning of the test and its behavior was manually recorded. The time spent in the center area was used as a measurement of anxiety and line crossing (defined as at least three paws in a square) was used as measurement of spontaneous locomotor activity [34]. The floor surface and the walls of the arena were thoroughly cleaned with 70% ethanol between tests.

2.3.3. Marble burying test (MBT)

The MBT was performed as described previously [35]. In brief, the test was performed in a plastic cage with approximately 4 cm of sawdust covering the floor. Twenty, colored glass marbles were evenly spaced (1 cm apart) over the floor on top of the sawdust. Each mouse was placed in the center of the marble-containing cage and tested for 15 min. The number of buried marbles (defined as those with at least two-third of the surface area under the sawdust) and the total duration of digging bouts were manually recorded for each animal [33].

2.3.4. Novelty suppressed feeding (NSF) test

The NSF test was performed according to the method described previously [35]. Briefly, animals were food-deprived for 24 h prior to the test. Testing was performed in a clear plastic ($40 \times 40 \times 20$ cm^3) box with the floor covered by 2 cm of sawdust. A single weighed pellet of food (standard chow) was placed on a white circular filter paper platform positioned in the center of the box. Mice were tested individually after placing them in the corner of the box for 10 min as described previously [35]. The latency to bite the food pellet was manually scored. Immediately afterwards, the mouse was returned to its home cage and the amount of food consumed during the subsequent free-feed 5 min was measured. For this test, the time taken (latency) to begin eating

food was measured as well as the amount of food consumed so as to control for any change in appetite as a potential confounding factor (home cage food intake). This was because antidepressants are known to affect appetite.

2.3.5. Tail suspension test (TST)

To determine the antidepressant like activity of BCP, mice were subjected to the TST as described previously [33]. In brief, for the conduction of the TST, the animals were individually suspended by the tail with a clamp using adhesive tape placed approximately 1 cm from the tip of the tail. The clamp was attached to a metal rod fixed 50 cm above the surface of a table covered with soft cloth. All animals were suspended for 6 min and the duration of behavioral parameters including immobility time (defined as the absence of any body or limb movements except for those caused by respiration) were manually recorded in seconds [35].

2.3.6. Forced swim test (FST)

The FST, which is a behavioral test for depression-like behavior often used to evaluate the effects of drug activity in rodents, was performed as originally described by Porsolt et al. with minor modifications [36]. Mice were placed individually into plastic transparent containers (18 cm diameter by 25 cm height) containing 15 cm of water at approximately 25 °C where they were expected to swim. At this depth, mice could not touch the bottom of the container with their tails or hind limbs. The duration of immobility (defined as the length of time in which the animal did not show escape responses) was manually scored during a 6 min session. The mouse was judged to be immobile when it remained in the water without struggling (passive floating) and was making only those movements necessary to keep its head above water [35,37]. Animals were then removed from the container and left to dry in a heated enclosure before they were returned to their home cages. The water was changed after each trial and the container cleaned thoroughly to get rid of the smell of the previous occupant.

2.4. Statistical analysis

For statistical comparisons, the software package IBM SPSS Statistics 20 (IBM Middle East, Dubai, UAE) was used. Mean ± SEM were calculated for each group. Dependent variables for each behavioral model were analyzed using one-way analyses of variance (ANOVA) with "treatment" as a between-subject factor. When relevant, post hoc analyses were performed by t-tests with Bonferroni corrections for multiple comparisons. $p < 0.05$ denotes a statistically significant difference.

3. Results

3.1. BCP produced an anxiolytic-like activity in C57BL/6 mice

3.1.1. Elevated plus maze (EPM)

The effects of BCP administration on anxiety-like behavior, characterized by increased open-arm exploration in the EPM test, are shown in Fig. 1. The one way-ANOVA test revealed a main effect of treatment on the percentage of time spent into the open arms (OA) ($F_{(2,22)} = 4.847$, $p = 0.018$) (Fig. 1A). Post hoc comparisons revealed a significant increase in the percentage of time spent by mice in the OA of the maze following the acute administration of exogenous BCP when compared to vehicle results ($p = 0.045$; DMSO-Oil vs. DMSO-BCP). However, pre-injection of the CB_2 receptor antagonist AM630 significantly abrogated the BCP effect ($p = 0.046$; DMSO-BCP vs. AM630-BCP) and no difference to vehicle results was observed ($p = 1.000$; DMSO-Oil vs. AM630-BCP). Similarly, and as depicted in Fig. 1B, there was a significant main effect of treatment on the number of entries into the OA ($F_{(2,22)} = 5.310$, $p = 0.013$). Post hoc evaluations indicated that an increased number of entries into the OA following acute BCP injection ($p = 0.030$; DMSO-Oil vs. DMSO-BCP) was reversed following AM630 injection ($p = 0.040$; DMSO-BCP vs. AM630-BCP). Furthermore, pretreatment with BCP increased the percentage of entries into the OA ($F_{(2,22)} = 10.383$, $p = 0.001$) (Fig. 1C). Post hoc evaluation revealed that the mice injected with BCP displayed more percentage of entries into the OA ($p = 0.002$; DMSO-Oil vs. DMSO-BCP) and that treatment with AM630 abrogated BCP-induced anxiolytic effect when compared with vehicle results ($p = 0.004$; DMSO-BCP vs. AM630-BCP). In contrast, no significant difference between vehicle and AM630-treated mice was found ($p = 1.000$; DMSO-Oil vs. AM630-BCP). Finally, and as depicted in Fig. 1D, measurements of general activity using the closed arm (CA) entries parameter did not differ significantly between any of the groups ($F_{(2,22)} = 2.634$, $p = 0.094$). Therefore, because no significant differences appeared in the number of CA entries between groups in the EPM test, the observed antianxiety-like behaviors of the mice receiving acute BCP injection are likely not attributable to differences in their locomotor activities.

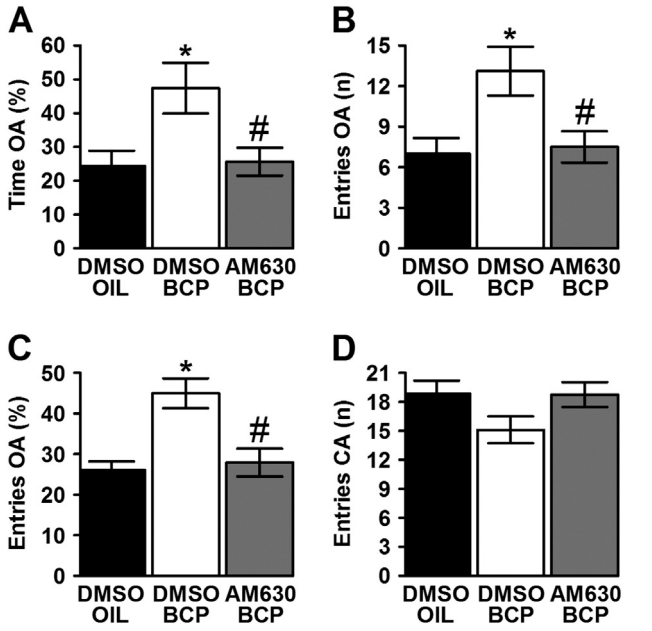

Fig. 1. Effects of acute BCP exposure on exploratory behavior on the elevated plus-maze (EPM) test. (A) percentage of time into the open arms (OA). (B) Number of entries into the OA. (C) Percentage of entries into the OA. (D) Number of entries into the closed arms (CA). Each bar represents mean ± SEM (n = 7–10). * denotes significant differences between DMSO-Oil and DMSO-BCP ($p < 0.05$). # denotes significant differences between DMSO-BCP and AM630-BCP ($p < 0.05$).

3.1.2. Open field (OF) test

The OF test was used to assess spontaneous locomotor activity and exploratory behavior among the mice receiving BCP injections and results are depicted in Fig. 2. The one-way ANOVA test revealed no significant effect of treatment on the number of line crossings ($F_{(2,22)} = 0.385$, $p = 0.685$) (Fig. 2A) suggesting no significant differences in locomotor activity (motor function) in the OF test among groups. However, there was a main effect of treatment on the time spent in the center area ($F_{(2,22)} = 7.759$, $p = 0.003$). In fact, and as depicted in Fig. 2B, mice receiving BCP injections displayed a significant increase in the time spent in the center of the open field ($p = 0.004$; DMSO-Oil vs. DMSO-BCP). However, AM630 pre-injection reversed the BCP-induced anxiolytic-effect ($p = 0.030$; DMSO-BCP vs. AM630-BCP). These findings suggest that BCP-treated mice subsequently produce exploration activities that are closely associated with anxiolytic-like behavior in the OF test.

3.1.3. Marble burying test (MBT)

To expand further the evaluation of the BCP anxiolytic-like effects, the MBT was also added and the results are shown in Fig. 3. The one-way ANOVA test revealed a significant effect of treatment on the

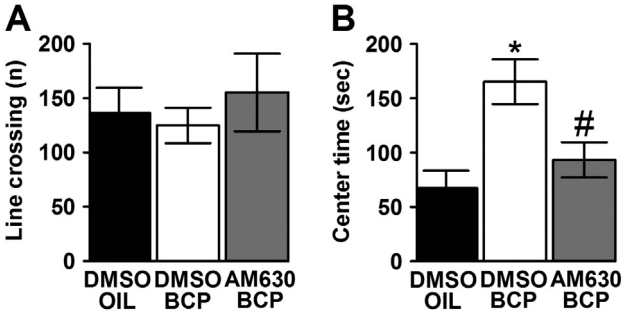

Fig. 2. Effects of acute BCP exposure on exploratory behavior on the open field (OF) test. (A) Number of line crossing. (B) Time spent in the center of the arena. Each bar represents mean ± SEM (n = 7–10). * denotes significant differences between DMSO-Oil and DMSO-BCP ($p = 0.004$). # denotes significant differences between DMSO-BCP and AM630-BCP ($p = 0.03$).

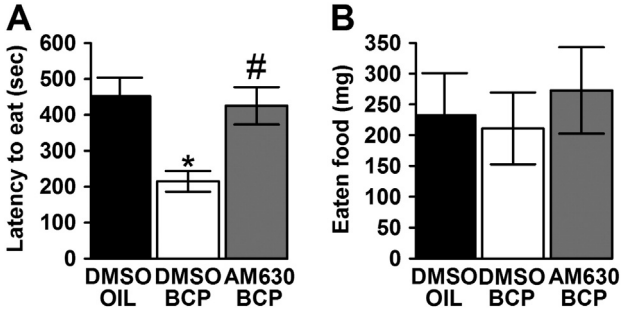

Fig. 4. Effects of acute BCP exposure on depression-like behavior on the novelty suppressed feeding (NSF) test. (A) Latency to initiate eating. (B) Amount of food consumed in the home cage. Each bar represents mean ± SEM (n = 7–10). * denotes significant differences between DMSO-Oil and DMSO-BCP ($p = 0.002$). # denotes significant differences between DMSO-BCP and AM630-BCP ($p = 0.005$).

number of buried marbles ($F_{(2,22)} = 9.134$, $p = 0.001$) (Fig. 3A). Post hoc evaluations revealed that the group treated with BCP had a reduced number of marbles buried by 55% when compared with the negative control ($p = 0.004$; DMSO-Oil vs. DMSO-BCP). In this experiment, the administration of AM630 reversed the reduction of the number of marbles buried induced by BCP ($p = 0.006$; DMSO-BCP vs. AM630-BCP). Interestingly, results from the animals in the AM630-pretreated group did not differ from those in the negative control group ($p = 1.000$; DMSO-Oil vs. AM630-BCP). Similarly, there was a significant effect of treatment on the time spent digging in the MBT ($F_{(2,22)} = 15.212$, $p < 0.000$) (Fig. 3B). Pair-wise comparisons revealed that BCP treatment reduced average digging time by approximately 70% ($p < 0.000$; DMSO-Oil vs. DMSO-BCP). However, results from the AM630 pre-treatment reversed BCP effect on this behavior ($p = 0.001$; DMSO-BCP vs. AM630-BCP) and showed no difference when compared to results from the vehicle group ($p = 1.000$; DMSO-Oil vs. AM630-BCP).

3.2. BCP produced an anti-depressant-like activity in C57BL/6 mice

As anxiety and depression are often co-morbid, the effects of BCP in three measurements of depression-like behavior were investigated: the NSF, the TST and the FST.

3.2.1. Novelty suppressed feeding (NSF) test

The effects of acute (50 mg/kg) BCP treatment were tested in C57BL/6 strain mice in the NSF test and results are depicted in Fig. 4. A one-way ANOVA test revealed a significant effect of treatment on the latency to feed ($F_{(2,22)} = 9.737$, $p = 0.001$) (Fig. 4A). The acute i.p. administration of BCP reduced the latency to feed in the NSF test with the magnitude of the effect being approximately 50% ($p = 0.002$; DMSO-Oil vs. DMSO-BCP). In contrast, acute AM630 pretreatment exerted an effect opposite to that of BCP by increasing the latency to feed ($p = 0.005$; DMSO-BCP vs. AM630-BCP). The feeding drive of each animal was assessed by returning it to its home-cage (familiar environment) immediately after the NSF test and weighing the amount of food pellets consumed over a period of 5 min. The one-way ANOVA test revealed that an acute administration of BCP alone or with AM630 did not affect the amount of home-cage food consumption ($F_{(2,22)} = 0.243$, $p = 0.787$) (Fig. 4B). These data clearly indicated that BCP produced an antidepressant-like effect with no incidence on appetitive behavior based on the lack of change in home-cage food consumption.

3.2.2. Tail suspension test (TST)

As depicted in Fig. 5A, there was a significant effect of treatment on immobility in the TST ($F_{(2,22)} = 6.324$, $p = 0.007$). Post hoc analysis revealed that the administration of BCP produced a significant reduction in the immobility time (approximately 51%) of animals in the TST ($p = 0.015$; DMSO-Oil vs. DMSO-BCP). Interestingly, the influence of treatment of mice with AM630 on the anti-immobility effect of BCP in the TST was also significant ($p = 0.027$; DMSO-BCP vs. AM630-BCP).

3.2.3. Forced swim test (FST)

The effects of BCP (50 mg/kg) on the immobility time in the FST can be seen in Fig. 5B. The one-way ANOVA test showed that there was a significant effect of treatment on this parameter ($F_{(2,22)} = 5.543$, $p = 0.011$). Post hoc evaluations indicated that mice subjected to the acute administration of exogenous BCP exhibited a significant anti-depressant -like behavior ($p = 0.021$; DMSO-Oil vs. DMSO-BCP) characterized by decreased duration of immobility (48%) during the FST compared to the results from vehicle-treated controls. However, AM630 pre-administration reversed the BCP-reduced time of immobility ($p = 0.047$; DMSO-BCP vs. AM630-BCP).

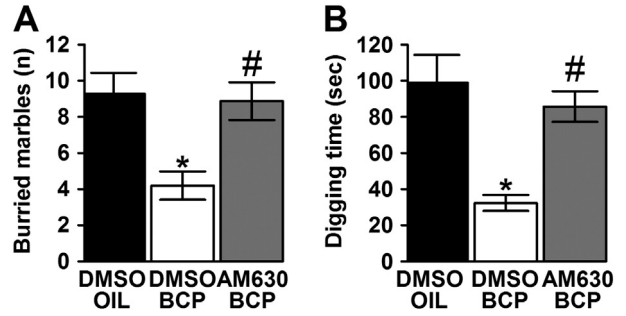

Fig. 3. Effects of acute BCP exposure on anxiety- and obsessive–compulsive-like behaviors on the marble burying test (MBT). (A) Number of buried marbles. (B) Time spent digging. Each bar represents mean ± SEM (n = 7–10). * denotes significant differences between DMSO-Oil and DMSO-BCP ($p < 0.005$). # denotes significant differences between DMSO-BCP and AM630-BCP ($p < 0.001$).

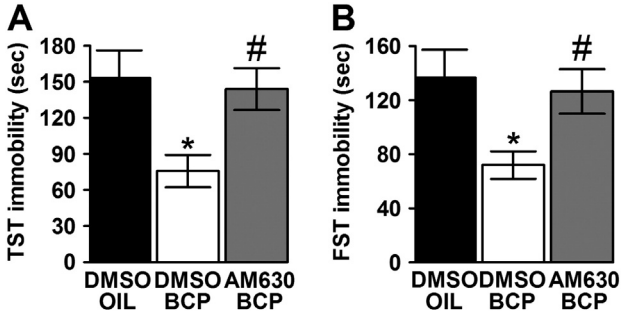

Fig. 5. Effects of acute BCP exposure on depression-like behavior on the tail suspension test (TST) and the forced swim test (FST). (A) Immobility time in the TST. (B) Immobility time in the FST. Each bar represents mean ± SEM (n = 7–10). * denotes significant differences between DMSO-Oil and DMSO-BCP ($p < 0.025$). # denotes significant differences between DMSO-BCP and AM630-BCP ($p < 0.05$).

4. Discussion

The present study demonstrates for the first time that BCP given systemically is effective in producing a significant anxiolytic- and antidepressant-like effects in most widely-used predictive animal models of anxiolytic (EPM, OF and MBT) and antidepressant activity (NSF, TST and FST). The study also demonstrates that the anxiolytic and antidepressant actions of BCP are mediated through CB_2 receptors. Evidence for this came from the CB_2 receptor antagonist AM630 abrogating the protective properties of BCP which provided evidence that the CB_2 receptor is involved in the regulation of anxiety- and depression-like behaviors.

Recent pharmacological and genetic findings indicate that the ECS comprising of endocannabinoid ligands and cannabinoid receptors (CB_1 and CB_2) is a target closely related to the regulation of mood disorders. However, with the CB_1 receptor antagonist, rimonabant (SR141716), the appearance of increased risk of anxiety, depression and suicidal thoughts has directed the development of other alternatives such as activation of CB_2 receptors [38–40] which is devoid of psychiatric adverse effects. Even though the CB_2 receptor has been considered the 'peripheral' cannabinoid receptor owing to its presence in the spleen and lymphocytes [41]. However, considerable functional and anatomical evidences demonstrate that CB_2 receptors are widely expressed in the CNS of rodents under normal conditions in several brain regions involved in a wide variety of physiological and pathological processes of the CNS including the regulation of emotional behaviors [for example, see [42–44]]. In the present study, the administration of BCP reduced the anxiety-like behavior in the EPM test. In addition, there was no significant difference in the number of closed-arm entries suggesting that the observed BCP-induced anxiolytic-like effect was not attributable to alterations of overall locomotor activity. Our findings are supported by Garcia-Gutiérrez and Manzanares, who reported that male mice over-expressing CB_2 receptors on a Swiss ICR congenic background showed a significant increase of the percentage of time spent in the open arms [45]. In the OF test the results of this study have shown that BCP was anxiolytic as it increases the time spent in the center area with no effect on total locomotor activity. This is in full agreement with a previous study where genetic over-expression of the CB_2 receptors had no effect on the total distance traveled but did increase the central distance traveled [45]. It should be emphasized, however, that Onaivi et al. reported that inhibition of the CB_2 receptor mRNA expression using bilateral microinjection of specific antisense oligonucleotides significantly increased the time spent in the open-arms of the EPM [46]. Further studies are needed to elucidate these discrepancies. In the MBT, the results from this study showed that BCP treatment reduced the number of buried marbles as well as the time spent digging, suggesting an anxiolytic-like activity that was abrogated upon AM630 pretreatment. Similar observations has been demonstrated in another study, wherein CB_2 receptor agonist GW405833 (100 mg/kg) significantly inhibited the burying behavior [47]. Considering the genetic relevance of the MBT in evaluating anxiolytic actions, the present study observations are clearly suggestive of the anxiolytic effects of BCP and can be correlated with the CB_2 receptors as AM630 abrogated the BCP effects.

Pharmacologically the conventionally used BZD class of anxiolytics is known to act through their binding on the interface of the $GABA_A$ receptor complex and promote the inhibitory actions of the GABA neurotransmitter in the CNS [for review see: [48–51]]. Recent studies suggest that administration of the CB_2 receptor agonist JWH13 causes suppression of GABAergic signaling in the hippocampus [52]. In another study, the deletion of the CB_2 receptor has shown decreased 5-HT_{2C} gene expression in the dorsal raphe and 5-HT_{2A} gene expression in the prefrontal cortex of CB_2 knockout mice [53]. It appears that there is an association between CB_2 receptors and serotonergic receptors however previous study report that BCP exhibits its anxiolytic effect independent of 5-HT and GABA receptors. This report convincingly indicates that a non-GABAergic and non-serotonergic mechanism is involved in the anxiolytic activity of BCP. Knowing the CB_2 receptor selectivity of BCP, we investigated BCP effects in the animal models of anxiety and depression using a battery of tests and to demonstrate the CB_2 receptor mediated activity we challenged animals with AM630, a selective CB_2 receptor antagonist. Based on the findings, the present study clearly demonstrates that the CB_2 receptors participate in the anxiolytic and antidepressant activity of BCP owing to its inherent cannabimimetic activity. This study corroborates with the previous study [30], wherein the authors demonstrated that a non-serotonergic and non-GABAergic mechanism involved in the anxiolytic activity of BCP. Thus the activation of CB_2 receptors seems to be a novel mechanism and targeting CB_2 receptors represent a novel pharmacological target for anxiolytic and antidepressants.

BCP administration produced an anti-depressant-like effect when assessed in models of depression (NSF, TST and FST) which are commonly used to identify new anti-depressant drugs. Thus, the fact that BCP administration is active in these tests support the hypothesis that this compound may play a role in the modulation of depression. In this study, BCP did not significantly alter motor activity in mice in the EPM and OF tests. Therefore, it is unlikely that the antidepressant-like effect of BCP observed in the FST is based on the stimulation of general motor activity.

The high lipophilicity of BCP suggests that it crossed the blood–brain barrier and acted at the CNS level [21]. In a quantitative structure–activity relationship study, BCP has been shown to possess a Log P value of 4.319 [54]. Also, AM630 is a potent and selective CB_2 receptor antagonist in the guinea pig brain [55]. It has a Ki of 32.1 nM at CB_2 and 165 times selectivity over CB_1 receptors in the in vitro and in vivo studies [21]. The Log P value of BCP and AM630 is more than 4, which indicates their lipophilicity, a favorable feature for brain penetration and highly suggestive of its CNS selectivity in coincide with previous studies [56,57].

Finally, the reversal of the BCP effect by AM630 clearly demonstrates that the activity of the central cannabinoid receptors plays a role in modulating the action of BCP in the anti-depressant and anxiolytic effect. In recent years emerging studies have demonstrated that CB_2 receptors play an important role in anxiety and stress-related disorders and suggest that targeting CB_2 receptors may have a potential role in anxiety and mood related disorders [45,58].

5. Conclusion

Considering the need for novel compounds that could improve conventional therapies as well as provide new agents targeting psychiatric disorders, the present study has clearly demonstrated the anxiolytic and anti-depressant effect of BCP and its underlying mechanism in a CB_2 receptor-dependent manner in rodents. The results also support the involvement of the CB_2 receptor in the regulation of emotional behavior and suggest that this receptor could be a relevant therapeutic target for the treatment of anxiety and depressive disorders.

Role of the funding source

The research was supported by the grants awarded from the National Research Foundation, United Arab Emirates to AB (grant no. 31M082) and SO (grant no. 31M099). The funder had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Author's contribution

AB was responsible for the study concept and design. All the authors contributed to the acquisition of animal data, performed the data analysis and helped in the interpretation of findings. AB and SO drafted the manuscript. All the authors provided critical revision of the

manuscript for important intellectual content and reviewed content and approved the final version for publication.

Disclosure/conflict of interest

The authors have no financial interests that might be perceived to influence the results or the discussion reported in this article.

Acknowledgments

The authors would like to acknowledge Mr. Mohamed Elwasila and Mr. Mohamed Shafiullah for their technical assistance and Dr. Mahmoud Hag Ali from the Central Animal Facility for his advice on veterinary care.

References

[1] Sansone RA, Sansone LA. Psychiatric disorders: a global look at facts and figures. Psychiatry (Edgmont (Pa : Township)) 2010;7:16–9.
[2] Hill MN, Gorzalka BB. The endocannabinoid system and the treatment of mood and anxiety disorders. CNS Neurol Disord Drug Targets 2009;8:451–8.
[3] Hill MN, Gorzalka BB. Impairments in endocannabinoid signaling and depressive illness. JAMA 2009;301:1165–6.
[4] Ashton CH, Moore PB. Endocannabinoid system dysfunction in mood and related disorders. Acta Psychiatr Scand 2011;124:250–61.
[5] Onaivi ES. Cannabinoid receptors in brain: pharmacogenetics, neuropharmacology, neurotoxicology, and potential therapeutic applications. Int Rev Neurobiol 2009;88:335–69.
[6] Riebe CJ, Wotjak CT. Endocannabinoids and stress. Stress 2011;14:384–97.
[7] Slipetz DM, O'Neill GP, Favreau L, Dufresne C, Gallant M, Gareau Y, et al. Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. Mol Pharmacol 1995;48:352–61.
[8] Abood ME, Martin BR. Molecular neurobiology of the cannabinoid receptor. Int Rev Neurobiol 1996;39:197–221.
[9] Bouaboula M, Poinot-Chazel C, Marchand J, Canat X, Bourrie B, Rinaldi-Carmona M, et al. Signaling pathway associated with stimulation of CB_2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. Eur J Biochem 1996;237:704–11.
[10] Moreira FA, Wotjak CT. Cannabinoids and anxiety. Curr Top Behav Neurosci 2010;2:429–50.
[11] Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, et al. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. Science 2005;310:329–32.
[12] Porter AC, Felder CC. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol Ther 2001;90:45–60.
[13] Piomelli D. The endocannabinoid system: a drug discovery perspective. Curr Opin Investig Drugs 2005;6:672–9.
[14] Mackie K. Cannabinoid receptors as therapeutic targets. Annu Rev Pharmacol Toxicol 2006;46:101–22.
[15] Marco EM, Garcia-Gutierrez MS, Bermudez-Silva FJ, Moreira FA, Guimaraes F, Manzanares J, et al. Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects. Front Behav Neurosci 2011;5:63.
[16] Costa E, Guidotti A. Benzodiazepines on trial: a research strategy for their rehabilitation. Trends Pharmacol Sci 1996;17:192–200.
[17] Mitler MM. Nonselective and selective benzodiazepine receptor agonists—where are we today? Sleep 2000;23(Suppl. 1):S39–47.
[18] Tong L, Shankar BB, Chen L, Rizvi R, Kelly J, Gilbert E, et al. Expansion of SAR studies on triaryl bis sulfone cannabinoid CB_2 receptor ligands. Bioorg Med Chem Lett 2010;20:6785–9.
[19] Thakur GA, Tichkule R, Bajaj S, Makriyannis A. Latest advances in cannabinoid receptor agonists. Expert Opin Ther Pat 2009;19:1647–73.
[20] Gertsch J, Pertwee RG, Di Marzo V. Phytocannabinoids beyond the Cannabis plant—do they exist? Br J Pharmacol 2010;160:523–9.
[21] Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, et al. Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A 2008;105:9099–104.
[22] Asgarpanah J, Kazemivash N. Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius* L. Chin J Integr Med 2013;19:153–9.
[23] McNeil M, Facey P, Porter R. Essential oils from the *Hyptis* genus—a review (1909–2009). Nat Prod Commun 2011;6:1775–96.
[24] Wadhams LJ, Birkett MA, Powell W, Woodcock CM. Aphids, predators and parasitoids. Novartis Found Symp 1999;223:60–7 (discussion 7–73).
[25] Fine PG, Rosenfeld MJ. The endocannabinoid system, cannabinoids, and pain. Rambam Maimonides Med J 2013;4:e0022.
[26] Ou MC, Hsu TF, Lai AC, Lin YT, Lin CC. Pain relief assessment by aromatic essential oil massage on outpatients with primary dysmenorrhea: a randomized, double-blind clinical trial. J Obstet Gynaecol Res 2012;38:817–22.
[27] Chen Y, Zhao YY, Wang XY, Liu JT, Huang LQ, Peng CS. [GC–MS analysis and analgesic activity of essential oil from fresh rhizoma of *Cyperus rotundus*]. Zhong Yao Cai 2011;34:1225–9.
[28] Mishra D, Bisht G, Mazumdar PM, Sah SP. Chemical composition and analgesic activity of *Senecio rufinervis* essential oil. Pharm Biol 2010;48:1297–301.
[29] Golshani S, Karamkhani F, Monsef-Esfehani HR, Abdollahi M. Antinociceptive effects of the essential oil of *Dracocephalum kotschyi* in the mouse writhing test. J Pharm Pharm Sci 2004;7:76–9.
[30] Galdino PM, Nascimento MV, Florentino IF, Lino RC, Fajemiroye JO, Chaibub BA, et al. The anxiolytic-like effect of an essential oil derived from *Spiranthera odoratissima* A. St. Hil. leaves and its major component, beta-caryophyllene, in male mice. Prog Neuropsychopharmacol Biol Psychiatry 2012;38:276–84.
[31] Bento AF, Marcon R, Dutra RC, Claudino RF, Cola M, Leite DF, et al. Beta-caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB_2 receptor activation and PPARgamma pathway. Am J Pathol 2011;178:1153–66.
[32] Horvath B, Mukhopadhyay P, Kechrid M, Patel V, Tanchian G, Wink DA, et al. Beta-caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. Free Radic Biol Med 2012;52:1325–33.
[33] Bahi A. Individual differences in elevated plus-maze exploration predicted higher ethanol consumption and preference in outbred mice. Pharmacol Biochem Behav 2013;105:83–8.
[34] Bahi A. Increased anxiety, voluntary alcohol consumption and ethanol-induced place preference in mice following chronic psychosocial stress. Stress 2013;16:441–51.
[35] Bahi A, Dreyer JL. Hippocampus-specific deletion of tissue plasminogen activator "tPA" in adult mice impairs depression- and anxiety-like behaviors. Eur Neuropsychopharmacol 2012;22:672–82.
[36] Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977;229:327–36.
[37] Bahi A, Mineur YS, Picciotto MR. Blockade of protein phosphatase 2B activity in the amygdala increases anxiety- and depression-like behaviors in mice. Biol Psychiatry 2009;66:1139–46.
[38] Moreira FA, Crippa JA. The psychiatric side-effects of rimonabant. Rev Bras Psiquiatr 2009;31:145–53.
[39] Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 2009;23:133–44.
[40] Le Foll B, Gorelick DA, Goldberg SR. The future of endocannabinoid-oriented clinical research after CB1 antagonists. Psychopharmacology (Berl) 2009;205:171–4.
[41] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.
[42] Maresz K, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP, et al. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB_1 on neurons and CB_2 on autoreactive T cells. Nat Med 2007;13:492–7.
[43] Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, et al. Discovery of the presence and functional expression of cannabinoid CB_2 receptors in brain. Ann N Y Acad Sci 2006;1074:514–36.
[44] Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, et al. Cannabinoid CB_2 receptors: immunohistochemical localization in rat brain. Brain Res 2006;1071:10–23.
[45] Garcia-Gutierrez MS, Manzanares J. Overexpression of CB_2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. J Psychopharmacol 2011;25:111–20.
[46] Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, et al. Functional expression of brain neuronal CB_2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. Ann N Y Acad Sci 2008;1139:434–49.
[47] Valenzano KJ, Tafesse L, Lee G, Harrison JE, Boulet JM, Gottshall SL, et al. Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology 2005;48:658–72.
[48] Phillis JW, O'Regan MH. The role of adenosine in the central actions of the benzodiazepines. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:389–404.
[49] Haefely WE. Pharmacology of the benzodiazepine receptor. Eur Arch Psychiatry Neurol Sci 1989;238:294–301.
[50] Suranyi-Cadotte BE, Bodnoff SR, Welner SA. Antidepressant–anxiolytic interactions: involvement of the benzodiazepine–GABA and serotonin systems. Prog Neuropsychopharmacol Biol Psychiatry 1990;14:633–54.
[51] Whiting PJ. The GABAA receptor gene family: new opportunities for drug development. Curr Opin Drug Discov Devel 2003;6:648–57.
[52] Morgan NH, Stanford IM, Woodhall GL. Functional CB2 type cannabinoid receptors at CNS synapses. Neuropharmacology 2009;57:356–68.
[53] Ortega-Alvaro A, Aracil-Fernandez A, Garcia-Gutierrez MS, Navarrete F, Manzanares J. Deletion of CB_2 cannabinoid receptor induces schizophrenia-related behaviors in mice. Neuropsychopharmacology 2011;36:1489–504.
[54] Chang HJ, Kim HJ, Chun HS. Quantitative structure–activity relationship (QSAR) for neuroprotective activity of terpenoids. Life Sci 2007;80:835–41.
[55] Hosohata K, Quock RM, Hosohata Y, Burkey TH, Makriyannis A, Consroe P, et al. AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain. Life Sci 1997;61:PL115–8.
[56] Choi IY, Ju C, Anthony Jalin AM, Lee da I, Prather PL, Kim WK. Activation of cannabinoid CB_2 receptor-mediated AMPK/CREB pathway reduces cerebral ischemic injury. Am J Pathol 2013;182:928–39.
[57] Elmann A, Mordechay S, Rindner M, Larkov O, Elkabetz M, Ravid U. Protective effects of the essential oil of *Salvia fruticosa* and its constituents on astrocytic susceptibility to hydrogen peroxide-induced cell death. J Agric Food Chem 2009;57:6636–41.
[58] Garcia-Gutierrez MS, Perez-Ortiz JM, Gutierrez-Adan A, Manzanares J. Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. Br J Pharmacol 2010;160:1773–84.