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Research report

Differential effects of CB1 receptor agonism in behavioural tests of unconditioned and conditioned fear in adult male rats

Jonathan J. Simone[a], Matthew R. Green[b], Travis E. Hodges[b], Cheryl M. McCormick[a,b,*]

[a] Department of Biological Sciences, Brock University, 500 Glenridge Ave., St. Catharines, ON L2S 3A1, Canada
[b] Department of Psychology, Brock University, 500 Glenridge Ave., St. Catharines, ON L2S 3A1, Canada

HIGHLIGHTS

- ACEA (CB1-R agonist) increased unconditioned and decreased conditioned fear.
- AM251 increased both unconditioned and conditioned fear in rats.
- Low dose ACEA had lower corticosterone than high ACEA and AM251 groups.

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ABSTRACT

We investigated the effects of the highly selective CB1 receptor agonist ACEA and the CB1 receptor antagonist/inverse agonist AM251 on two behavioural tests of unconditioned fear, the elevated plus maze (EPM) and open field test (OFT), as well as on the recall and extinction of a conditioned auditory fear. Both ACEA and AM251 increased anxiety-like behaviour in the EPM and OFT. There was no effect of either drug on recall of the conditioned fear, and ACEA enhanced and AM251 impaired fear extinction. Further, though both the low (0.1 mg/kg) and high (0.5 mg/kg) dose of ACEA facilitated fear extinction, the low dose attenuated, and the high dose potentiated, fear induced corticosterone release suggesting independent effects of the drug on fear and stress responses. Although the extent to which cannabinoids are anxiogenic or anxiolytic has been proposed to be dose-dependent, these results indicate that the same dose has differential effects across tasks, likely based in differences in sensitivities of CB1 receptors to the agonist in the neural regions subserving unconditioned and conditioned fear.

1. Introduction

The endogenous cannabinoid system (ECS) is a retrograde messenger system comprised of cannabinoid type 1 (CB1) and type 2 (CB2) receptors, the endogenous ligands arachidonylethanolamine (AEA) and 2-arachidonyl glycerol (2-AG), as well as the enzymes responsible for the synthesis and degradation of these ligands, notably diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL) for 2-AG and fatty acid amide hydrolase (FAAH) for AEA [1,2]. Although CB1 and CB2 receptors are both $G_{i/o}$-coupled G-protein coupled receptors (GPCR) with affinities for the same endogenous ligands [3,4], their spatial distribution within an organism supports the idea that they participate in different physiological processes. CB1 receptors are the primary neuronal cannabinoid receptor, with widespread distribution throughout the brain, particularly in cortical and limbic structures such as the medial prefrontal cortex, the hippocampus, and the amygdala [5,6]. CB2 receptors, on the other hand, are expressed primarily in the periphery, with the greatest expression found in immune tissues [4,6]. Evidence does exist, however, for the expression of CB2 receptors in the brain [7,8].

Given the localization of cannabinoid receptors in the brain's emotional circuitry, it is not surprising that the ECS has emerged as a key regulator of behavioural fear responses in mammals. Pharmacological enhancement of ECS signalling via inhibition of endocannabinoid metabolism and reuptake elicits anxiolytic effects in several behavioural tests of fear [9,10]. Studies using direct agonists of CB receptors, however, have generated mixed results; systemic administration of exogenous cannabinoid agonists has been shown to both increase [11,12] and decrease [13–15]

* Corresponding author at: Centre for Neuroscience, Brock University, 500 Glenridge Ave., St. Catharines, ON L2S 3A1, Canada. Tel.: +1 905 688 5550x3700; fax: +1 905 688 6922.
E-mail addresses: js09vp@brocku.ca (J.J. Simone), mg06mc@brocku.ca (M.R. Green), th12iw@brocku.ca (T.E. Hodges), cmccormick@brocku.ca, cmccormi@brocku.ca (C.M. McCormick).

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behavioural fear in rats. The effects on fear are often dose- and task-specific, and few studies involved more than one type of behavioural measure; the use of different endpoints may help parse out the functional role of these receptors in normative and pathological fear.

Recently, Fogaca and colleagues [16] found different effects of the phytocannabinoid cannabidiol (CBD) on unconditioned and conditioned fear in male rats. Specifically, CBD injected directly into the prelimbic division of the prefrontal cortex increased anxiety-like behaviours in the elevated plus maze, but decreased the recall of a conditioned fear of context [16]. They also demonstrated a role of $5HT_{1A}$ receptors in the contrasting behavioural effects of CBD, but the contributions of CB receptors were not investigated. Although it is possible that the behavioural effects of CBD are independent of CB receptor activity because CBD is highly promiscuous, CBD does have affinity (albeit low) for both CB1 and CB2 receptors and can indirectly activate CB receptors through the inhibition of AEA hydrolysis and re-uptake [17,18]. Thus, a role of CB receptors in the behavioural effects of CBD cannot be ruled out. Nevertheless, the contrasting effects of CBD on different behavioural measures of fear within the same neural region highlights the value of including a variety of endpoints for analysis within an experiment.

We investigated whether such contrasting effects on conditioned and unconditioned fear in adult male rats as reported by Fogaca and colleagues [16] would be observed after administration of the highly selective CB1 receptor agonist Arachidonyl 2′-Chloroethylamide (ACEA) or the CB1 receptor selective antagonist/inverse agonist AM251. We used two tests of unconditioned anxiety that also allowed measurement of locomotor activity. In our test of conditioned fear, we assessed generalized fear (fear in a new context rather than fear of the conditioned context), fear of a conditioned cue (tone), and extinction of fear to the conditioned cue. Further, as the endocannabinoid system modulates hypothalamic-pituitary-adrenal axis responses to stressors in rodents [19], we also measured plasma corticosterone concentrations after exposure to fear recall and extinction testing. We predicted reduced fear behaviours and reduced corticosterone release in response to fear after systemic injections of ACEA, and increased fear behaviours and corticosterone release after systemic injections of AM251.

2. Methods

2.1. Animals

Male Long–Evans rats ($N = 36$) were obtained from Charles River (St. Constant, Quebec) at ~60 days of age. Rats were housed in pairs and maintained under a 12 h light–dark cycle (lights on at 08:00 h) with food and water available ad libitum. Use of animals in this experiment was approved by the Brock University Institutional Animal Care and Use Committee and was carried out in adherence to the Canadian Council of Animal Care and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) guidelines. All procedures were conducted between 9:00 and 17:00 h.

2.2. Drugs

The highly selective CB1 receptor agonist Arachidonyl 2′-Chlorethylamide (ACEA) (Cayman Chemical, USA) and the CB1 receptor antagonist/inverse agonist AM251 (Tocris Bioscience, USA) were diluted in 1:1:18 mixture of DMSO, Tween-80, and 0.9% saline. The vehicle was a 1:1:18 mixture of DMSO, Tween-80, and 0.9% saline in the absence of ACEA or AM251. For the elevated plus maze test, ACEA was administered at a dose of 0.5 mg/kg. As this dose unexpectedly produced an anxiogenic response (see Section 3.1), a dose of 0.1 mg/kg was added for the open field test and recall of conditioned fear and extinction testing. A dose of 3 mg/kg was selected for AM251 based on previous studies [20] and was used for all experiments. All injections were administered i.p. at a volume of 1 mL/kg 30 min prior to behavioural testing, and there was a washout period of 48 h between injections for each behavioural test.

2.3. Behavioural testing

2.3.1. Elevated plus maze

The elevated plus maze is a test of unconditioned avoidance behaviour that has been well-validated as a measure of anxiety in rodents [21]. The apparatus consisted of two open arms and two closed arms extended from a common central platform 80 cm in height. The maze was constructed of grey plastic and was situated in the centre of the testing room. 30 min after injection, rats were placed into a closed arm of the maze, and testing lasted for 5 min. Testing took place under low illumination, and behaviour was recorded by an overhead camera. Behaviour on the maze was scored by an observer blind to experimental condition. An arm entry was recorded when the two front paws of the animal were in the arm. Behaviours scored were the time spent on the open arms, time spent on the closed arms, time in the centre space, number of entries into an open arm, and number of entries into a closed arm. The percentage of time spent on the open arms of the maze and the number of entries onto an open arm are the standard measures of anxiety-like behaviour in this test [22]. Percent time on the open arm was calculated as follows: Time on Open arm/300 s × 100. The number of entries onto the closed arms of the maze was used as a measure of locomotor activity [23,24]. The maze was cleaned with Virox disinfectant between each animal. To minimize effects of separation of cage partners on performance, cage partners were tested 2 h apart to allow recovery from any stress attributable to the cage partner's absence of approximately 7 min.

2.3.2. Open field test

Rats were tested in an open field test 48 h after the elevated plus maze test. The open field test, like the elevated plus maze, involves the conflict between motivation to explore and fear of open spaces [25]. Although the open field test is commonly used to measure locomotor activity (e.g., distance travelled), latency to enter and time spent in the centre of an open field are validated measures of anxiety (longer latencies, less time in centre) in rats [21]. The open field consisted of white open-top melamine arenas (58 cm × 58 cm × 58 cm) illuminated indirectly by red light to attenuate anxiety related to bright illumination. The testing room contained four open field arenas, allowing for four animals (two pairs of cage partners) to be tested simultaneously. The test session was 30 min in duration, and locomotor activity was recorded with a Sony digital video camera mounted from the ceiling and connected to a computer tracking system (Smart; Panlab, Spain) that measured distance travelled in centimeters, as well as the percentage of time spent in the centre of the test arena (12 cm away from any wall). The maze was cleaned with Virox disinfectant after each session. As 0.5 mg/kg ACEA produced anxiety-like behaviours in the EPM, four rats from the AM251 group and four rats from the ACEA-0.5 group were reassigned to a lower dose of 0.1 mg/kg ACEA.

2.3.3. Fear conditioning

Rats were trained and tested in one of four identical cages (30 cm × 37 cm × 25 cm), each situated in a ventilated, sound-attenuating chamber with a small window (8 cm diameter) on the door that was covered by a red Plexiglas pane, and containing an LED light source on the rear wall (all equipment from Panlab,