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Research report

Cannabinoids & Stress: Impact of HU-210 on behavioral tests of anxiety in acutely stressed mice

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HIGHLIGHTS

- We examined the effects of HU-210 on anxiety behaviors in an acute stress mouse model.
- In stressed mice, both high and low doses of cannabinoids were anxiogenic.
- Locomotor deficits appeared at high doses of HU-210, but only in stressed mice.
- HU-210 produces different behavioral phenotypes in stressed and unstressed subjects.
- A summation hypothesis is proposed to explain this phenomenon.

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ABSTRACT

Anxiety disorders are one of the most prevalent classes of mental disorders affecting the general population, but current treatment strategies are restricted by their limited efficacy and side effect profiles. Although the cannabinoid system is speculated to be a key player in the modulation of stress responses and emotionality, the vast majority of current research initiatives had not incorporated stress exposure into their experimental designs. This study was the first to investigate the impact of exogenous cannabinoid administration in an acutely stressed mouse model, where CD1 mice were pre-treated with HU-210, a potent CB1R agonist, prior to acute stress exposure and subsequent behavioral testing. Exogenous cannabinoid administration induced distinct behavioral phenotypes in stressed and unstressed mice. While low doses of HU-210 were anxiolytic in unstressed subjects, this effect was abolished when mice were exposed to an acute stressor. The administration of higher HU-210 doses in combination with acute stress exposure led to severe locomotor deficits that were not previously observed at the same dose in unstressed subjects. These findings suggest that exogenous cannabinoids and acute stress act synergistically in an anxiogenic manner. This study underlies the importance of including stress exposure into future anxiety-cannabinoid research due to the differential impact of cannabinoid administration on stressed and unstressed subjects.

1. Introduction

According to the World Health Organization's mental health survey, anxiety disorders are consistently the most prevalent class of mental disorder affecting the general population, averaging a lifetime prevalence of 16% across 17 participating countries [1]. Despite the need for adequate anxiolytic treatment options, it is generally believed that existing treatment options, including $GABA_A$ agonists, monoamine oxidase (MAO) inhibitors and selective serotonin reuptake inhibitors (SSRIs), are restricted by their limited efficacy and problematic side effect profiles [2]. The lack of knowledge surrounding the precise cellular and molecular mechanisms underpinning the psychopathologies characteristic of anxiety disorders is one of the contributing factors to the current constraints on the development of newer, more efficacious therapeutic interventions.

The cannabinoid (CB) system has attracted a lot of attention as a potential neuromodulatory system involved in the regulation of stress and emotionality. Although cannabis was historically

Abbreviations: CB, cannabinoid; HU-210, 1,1-dimethylheptyl-11-hydroxytetrahydrocannabinol; OFT, open field test; EPM, elevated-plus maze; FS, forced swimming test; CB1R, cannabinoid type-1 receptor.

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used as a treatment for anxiety in ancient Indian medicinal practices over 2600 years ago [3], it wasn't until millennia later that empirical research provided evidence for CB modulation of anxiety. The increased popularity of recreational marijuana use prompted numerous research initiatives in the 1960s, one of which lead to the discovery of one of the main psychoactive agents, (−)-trans-Δ9-tetrahydrocannabinol (Δ^9-THC) [4]. Further studies showed that Δ^9-THC mediated its psychoactive effects through a novel receptor, later deemed the cannabinoid type-1 receptor (CB1R) [5]. Subsequently, CB1Rs were identified in key brain regions associated with stress response regulation, including the pituitary gland, hippocampus, pre-frontal cortex and amygdala [6,7]. Furthermore, these receptors were highly expressed in the pre-synaptic terminals of GABA inter-neurons [8] and low, but significant, levels were found at pre-synaptic glutamatergic terminals [9]. The anatomical and cellular localization of these receptors make the eCB system a prime candidate for a neuromodulator of anxiety circuitry, as it is commonly accepted that excessive or inappropriate excitatory glutamate activity within anxiety-related brain circuits is an underlying factor in the pathology of anxiety disorders [10].

It is generally accepted that CBs themselves modulate anxiety in a biphasic manner, where low doses are anxiolytic and high doses increase anxiety-related behavior. However, the vast majority of the CB-anxiety studies had not incorporated stress exposure into their experimental designs. In most cases, the anxiolytic and anxiogenic potentials of a given synthetic CB are measured by comparing the exhibition of anxiety-related behaviors of treated and non-treated subjects in an unstressed animal model. One exception is a recent study that sought to investigate the effect of cannabinoid pre-treatment in a rat model of chronic stress. The authors reported that the same dose of cannabinoids has distinct effects on anxiety-related behaviors in the elevated-plus maze (EPM) in stressed and unstressed subjects. Specifically, unstressed animals exhibited the classic bidirectional responses to high and low doses of HU-210 treatment, whereas chronically stressed rats treated with both high and low doses of HU-210 elicited anxiogenic behavioral phenotypes [11].

These findings emphasize the need for the incorporation of stressors in cannabinoid-anxiety research. In most cases, the anxiolytic and anxiogenic potentials of a given synthetic CB are measured by comparing the exhibition of anxiety-related behaviors of treated and non-treated subjects in an unstressed animal model. Although the existing experimental design accurately measures changes in anxiety-related behaviors, the model lacks face and external validity without the inclusion of a stressor. In order to address this gap in the literature, the present study aims to investigate the effects of CB pre-treatment on anxiety-related behaviors in acutely stressed mice.

2. Materials and methods

2.1. Experimental subjects

Experiments were conducted on 7-week-old male CD1 mice (30–40 g). The mice were housed in cages of 4 under standard laboratory conditions (ad libitum food and water, 12 hour light–dark cycle [7 AM–7 PM]). All experimental subjects were handled in accordance with the guidelines of the Canadian Council on Animal Care and the protocols approved by the Animal Care Committee of the Institute of Mental Health Research (Ottawa, Ontario).

2.2. Drugs and drug administration

HU-210, a potent CB1R agonist, was obtained from Sigma (St. Louis, USA). The crystalline form of the drug was dissolved in a vehicle of 1:1:18 of dimethyl sulfoxide (DMSO): Tween-80: 0.9% saline. HU-210 treatment was administered at $v = 0.005$ ml/g at four doses: 50, 25, 10 and 5 μg/kg. A vehicle injection was also administered at the same volume as a control. All treatments were received via intra-peritoneal (IP) injections with a 26-gage ½-inch needle.

2.3. Behavioral testing apparatuses

Three behavioral tests were used in the current study. A Forced Swimming test (FS) was administered as an acute stressor, whereas the Open Field Test (OFT) and Elevated-Plus Maze (EPM) were employed to investigate changes in anxiety-related behaviors across treatment conditions. The procedures and protocols for the FS, OFT and EPM have been previously reported [12–14], respectively.

2.4. Experimental approach

Mice were randomly assigned to a stressed or non-stressed paradigm. In the stressed condition, mice were pre-treated with HU-210 or its vehicle and habituated for 30 min before being exposed to an acute stressor, a 10-min FS test. Subsequently, an hour was given to rest before behavioral testing. The mice were then placed in a corner of the OFT arena (facing the center) and left to explore for 5 min. The time spent in the center region was manually scored, while ambulatory movement was recorded by an Opto-Varimex-Minor (Columbus instruments). Immediately after the OFT, the mice were transferred to the EPM, where they were placed on the central square (facing an open arm) and tested for 5 min. The time spent in the open arms, central square and closed arms were manually scored.

In the unstressed condition, mice were pre-treated with HU-210 or its vehicle and habituated for 30 min before behavioral testing in the OFT and EPM. In addition, non-treated mice were run through both the stressed and unstressed experimental paradigms as controls.

All testing apparatuses were cleaned with 70% ethanol between experimental subjects.

2.5. Exclusion criterion

The literature has well-documented cannabinoid alteration of locomotor activity [16]. Seeing as our measures of anxiety depend on the mice's ability to move from one region to another within the apparatuses, an exclusion criterion was implemented. If the ambulatory movement of a mouse was ±2 standard deviations from the mean of a given group, its data was omitted from future statistical analyses.

2.6. Statistical analyses

Statistics were computed on SPSS (version 22) and significance was set at $p < 0.05$. Two-way *t*-tests were completed with Welch's Corrections, whereas multivariate and one-way ANOVAs with Fisher's LSD tests.

3. Results

3.1. The acute stress paradigm successfully induced an anxiogenic behavioral phenotype without locomotor deficits

First, the efficacy of the acute stress paradigm used in this series of experiments was evaluated (Fig. A.1). As expected, a series of t-tests revealed that untreated stressed mice spent significantly less time in the center region of the OFT arena ($^*p = 0.0058$) and the open arm of the EPM ($^*p = 0.0099$) when compared to unstressed