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# Enhancement of anxiety-like responsiveness to the cannabinoid $CB_1$ receptor agonist HU-210 following chronic stress

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**Abstract**

The effect that chronic unpredictable stress had on the anxiety-like response elicited by the cannabinoid receptor agonist HU-210 [3-(1,1-dimethylheptyl)-(-)-11-hydroxy-delta8-tetrahydrocannabinol] in the elevated plus maze was investigated here. Male Long–Evans rats were either unstressed or were subjected to a 21-day regimen of chronic unpredictable stress, and subsequently were subdivided into three testing groups (vehicle, 10 and 50 μg/kg of HU-210) and tested on the elevated plus maze. Results demonstrated that in unstressed animals, a low dose of HU-210 induced an anxiolytic response, whereas a high dose induced an anxiogenic response. Further, in stressed animals both the low and the high doses of HU-210 induced anxiogenic responses. These findings suggest that chronic stress enhances either cannabinoid receptor responsivity or one of the interacting systems implicated in emotional states.


*Keywords:* Cannabinoid; Anxiety; Elevated plus maze; Chronic stress; (Rat)

## 1. Introduction

The neural endogenous cannabinoid system is composed of a cannabinoid $CB_1$ receptor ($CB_1$) and two endogenous arachidonate-derived ligands, anandamide and 2-arachidonylglycerol. The endocannabinoid system is a neuromodulatory system that regulates excitatory and inhibitory neurotransmission in the brain, and is thought to play a critical role in the regulation of multiple physiological processes (Freund et al., 2003). There is growing interest in the notion that the endocannabinoid system plays a physiological role in the regulation of emotional states, such as anxiety. The basis for this interest has multiple roots: First, the $CB_1$ receptor is located, neuronally, in stress responsive circuits that are crucial to the formation and expression of anxiety, such as the prefrontal cortex, amygdala and hypothalamus (Herkenham et al., 1991); second, acute restraint stress has been shown to elevate anandamide and 2-arachidonylglycerol synthesis in the limbic forebrain, suggesting that EC activity may play a role in modulating stress and anxiety (Hillard and Patel, personal communication); third, pharmacological and genetic manipulations of the EC system differentially alter behavioural responsiveness to stress and anxiety in animals (Haller et al., 2002; Martin et al., 2002). For example, mice lacking the $CB_1$ receptor demonstrate enhanced anxiety in a variety of paradigms (Haller et al., 2002; Martin et al., 2002), however acute blockade of $CB_1$ receptors by SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] can be either anxiogenic (Navarro et al., 1997) or anxiolytic (Haller et al., 2002). Moreover, high doses of cannabinoid agonists elicit anxiogenic responses (Marin et al., 2003; Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996) whereas mild pharmacological enhancement of EC activity appears to elicit anxiolytic effects (Berrendero and Maldonado, 2002; Kathuria et al., 2003; Rodriguez de Fonseca et al., 1996). Parsimoniously, these data suggest that large increases or decreases in endocannabinoid signaling are anxiogenic, whereas mild

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changes in endocannabinoid signaling can result in either anxiogenesis or anxiolysis, a phenomenon likely due to the differences in strain of animal, testing paradigm or the environmental conditions.

Environmental conditions are likely to play a crucial role in determining the effects of altered endocannabinoid activity on anxiety in animals. For example, social isolation and food deprivation for 24 h have been shown to potentiate the hyper-reactive state induced by acute cannabinoid administration in the rat (MacLean and Littleton, 1977). Accordingly, it also appears that environmental conditions modulate the emotional responses to cannabis in humans. Recreational cannabis consumption, where low doses of cannabis are consumed in familiar, comfortable environments, typically results in positive responses such as enhanced euphoria and a reduction in anxiety (Hollister, 1986). However, cannabis is also known to elicit negative reactions in humans, most commonly manifested as increases in panic, anxiety and paranoia, which commonly occur in response to high doses of consumption (Hollister, 1986) or in environments that are unfamiliar or stressful (Gregg et al., 1976; Talbott and Teague, 1969).

Thus, increases in acute environmental stress in rats (MacLean and Littleton, 1977) and humans (Gregg et al., 1976) potentiate the anxiety-like response elicited by cannabinoids. Furthermore, in humans, chronic exposure to stressful conditions exacerbates the likelihood of an aversive response to cannabis (Talbott and Teague, 1969). The aim of this experiment is to examine how chronic exposure to unpredictable, environmental stress will affect anxiety responses elicited by the acute administration of a cannabinoid $CB_1$ receptor agonist, as measured in the elevated plus maze.

## 2. Methods

### 2.1. Subjects

Subjects were 300-g male Long–Evans rats (70 days of age at onset of experiment) housed in groups of three in triple mesh wire cages. Colony room temperature was maintained at 21±1 °C, and lighting was maintained on a reverse 12-h light/dark cycle (lights off at 0900 h). Food (Purina Rat Chow) and tap water were available ad libitum, except during deprivation in the stress regimen.

### 2.2. Drugs

HU-210 [3-(1,1-dimethylheptyl)-(−)-11-hydroxy-$\Delta^8$-tetrahydrocannabinol], a potent and non-selective $CB_1$ receptor agonist, was obtained from Tocris-Cookson (Bristol, UK) and dissolved in a vehicle of 8:1:1 of 0.9% saline: dimethyl sulfoxide (DMSO): Tween 80. HU-210 was administered at doses of 10 and 50 μg/kg in a volume of 1 ml/kg. A corresponding vehicle injection was also administered to control rats at the same volume. All injections were performed intraperitoneally with a 26-gauge 1/2-in. needle.

### 2.3. Apparatus

The elevated plus maze consists of two open arms (50×12.5 cm) and two enclosed arms (50×12.5×50 cm) which extend from a common middle platform (12.5×12.5 cm). The apparatus was constructed from wood, with all walls and platforms painted black. The extending arms were elevated above the ground at a height of 60 cm by four pedestals on which each of the extended arms was resting.

### 2.4. Procedure

Animals were randomly divided into two experimental groups; chronic stress or cage control condition. Chronically stressed animals were subjected to a 3-week regimen of rotating stressors. The stress paradigm had animals subjected to three varying mild stressors a day. Stressors consisted of: (1) 30 min of tube confinement; (2) 5 min of forced swim; (3) 12-h food and/or water deprivation; (4) 30 min of social crowding and exposure to white noise and stroboscopic illumination; (5) 3 h of random cage rotation (animal's cage assignments are randomly rotated so that animals are removed from their dominance hierarchy and put in a new situation where they fight to re-establish dominance); (6) 12 h of social isolation. All short-term stressors (3 h or less) were performed during the dark cycle and the 12-h stressors spanned the light cycle. Cage control animals were handled daily to habituate them to human contact and control for any stress induced by handling.

All behavioural testing was conducted under dim illumination during the middle third of the dark cycle. Twelve hours following the last stressor (12-h food and water deprivation), both stress and non-stress groups were randomly subdivided into three treatment groups (all *n*'s 6–8/group): (1) vehicle; (2) 10 μg/kg of HU-210; (3) 50 μg/kg of HU-210. Thirty min after receiving treatment, all subjects were placed on the central platform of the elevated plus maze facing an open arm. Each animal was given a 5-min trial, during which both the frequency of entries into and total time spent in each of the open and closed arms was scored by observers blind to the condition of the animal. Arm entries were defined by at least three of the rats' paws entering an area and as animals left a closed to an open arm, or vice versa, they were considered in the prior arm until all three paws had entered the new arm, thus any time in the central area was counted as time in the previous arm. After individual rats finished their trial in the apparatus, the walls and platform floors were wiped with a 1% hibitane solution and dried to remove any scent trail that the subject might have left. Five animals from the vehicle group of each condition were euthanized in a $CO_2$ chamber (under ethical procedures of the University of British Columbia Ethics

Committee and Canadian Council of Animal Care) following their trial in the elevated plus maze and had their adrenal glands removed.

### 2.5. Statistics

Behavioural data were analyzed using a univariate analysis of variance, with condition and drug dose as fixed factors. Post hoc analyses were performed using Newman–Keuls tests. Adrenal weights were compared with a *t*-test. Significance levels were set at an alpha value of 0.05.

## 3. Results

Results from this experiment demonstrated that in non-stressed rats there was a bi-directional effect of HU-210 on anxiety-like behaviour that was dose dependent, and that this effect was altered following stress. Analysis of data revealed that there was a significant interaction between stress treatment and drug dose on time spent in open arms $F(2,37)=16.282$, $P<0.001$. Post hoc analysis demonstrated that in the non-stressed animals, 10 μg/kg of HU-210 increased time spent in open arms ($P=0.035$) and 50 μg/kg of HU-210 decreased time spent in open arms ($P<0.001$). Furthermore, in the stressed animals, both the 10- and the 50-μg/kg doses of HU-210 decreased time spent in the open arms (both $P<0.001$). In animals treated with vehicle alone, there was no effect of stress ($P>0.05$). However, there was a significant difference in time spent in the open arms between the stressed and non-stressed animals following a dose of 10 μg/kg of HU-210 ($P<0.001$). There were no differences between the stressed and non-stressed animals following a dose of 50 μg/kg HU-210 ($P>0.05$). Data for time spent in open arms are presented in Fig. 1A.

For frequency of entries into the open arms, there was a significant interaction between stress treatment and drug dose $F(2,37)=7.311$, $P=0.002$. Post hoc analysis demonstrated that in the non-stressed animals, 10 μg/kg of HU-210 increased the frequency of open arm entries ($P<0.01$), however there was no difference between vehicle and 50 μg/kg of HU-210 on frequency of open arm entries ($P>0.05$). In the stressed condition, neither 10 nor 50 μg/kg of HU-210 induced any significant change in the frequency of open arm entries compared to the vehicle treated group (both $P>0.05$). Comparison between stressed and non-stressed conditions on entries into open arms demonstrated that there was no difference between vehicle ($P>0.05$) or 50 μg/kg of HU-210 ($P>0.05$) in either group. There was however a significant reduction in the frequency of open arm entries following 10 μg/kg of HU-210 in the stressed group relative to the non-stressed group ($P<0.001$). Data for frequency of open arm entries are presented in Fig. 1B.

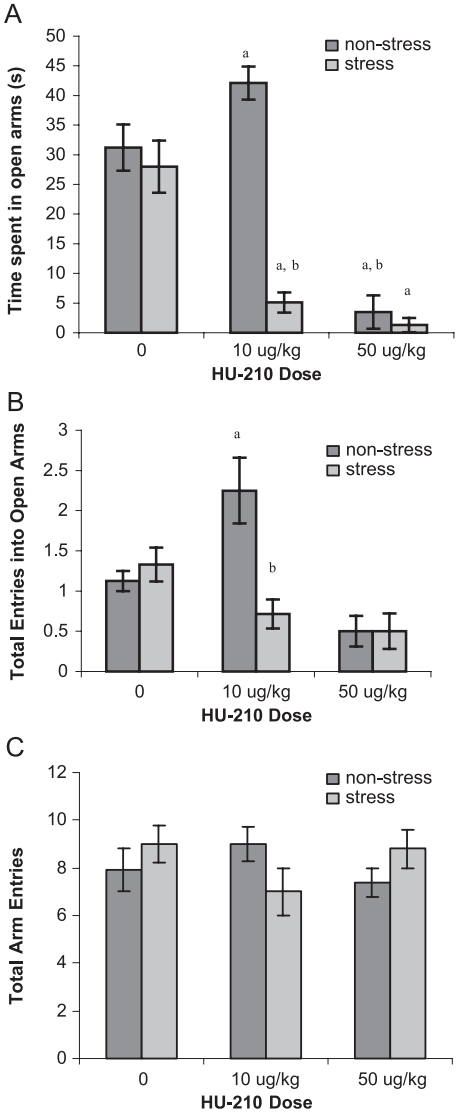


Fig. 1. (A) The effect of stress and dose of HU-210 on time spent in open arms. Significant differences from same group vehicle at $P<0.05$ are denoted by [a]. Significant differences from non-stressed 10 μg/kg of HU-210 group at $P<0.05$ are denoted by [b]. (B) The effect of stress and dose of HU-210 on entries into open arms. Significant differences from same group vehicle at $P<0.05$ are denoted by [a]. Significant differences from non-stressed 10 μg/kg of HU-210 group at $P<0.05$ are denoted by [b]. (C) The effect of stress and dose of HU-210 on total arm entries.

Analysis demonstrated that there was no significant interaction between stress condition and drug dose on total arm entries [$F(2, 37)=2.748$, $P>0.05$]. Furthermore, there were no main effects of stress on total arm entries [$F(2,37)=0.774$, $P>0.05$], or main effect of drug dose on total arm entries [$F(2,37)=0.153$, $P>0.05$]. Data for total arm entries are presented in Fig. 1C.

Comparison of adrenal weights between the stressed and non-stressed condition illustrated that adrenal weights were significantly elevated in the stressed condition [$t(8)=-3.155$, $P=0.014$]. Adrenal weights in the stressed condition were $42.7\pm4.4$ mg compared to $25.1\pm3.4$ mg in the control condition.

## 4. Discussion

In non-stressed rats, a low dose of the cannabinoid agonist HU-210 elicited an anxiolytic response in the elevated plus maze, and a high dose induced an anxiogenic response. This result is in line with previous research demonstrating that mild enhancement of endocannabinoid signaling induces an anxiolytic response in other paradigms (Berrendero and Maldonado, 2002; Kathuria et al., 2003), and that high doses of $CB_1$ receptor agonists induce anxiety-like responses (Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996). However, in stressed rats, the low dose of HU-210 induced an anxiogenic response that was no different than that elicited by a high dose of HU-210. These findings can not be attributed to cannabinoid or stress-induced changes in motor functioning as they were no differences between any of the groups on total arm entries. The lack of effect of chronic unpredictable stress alone on anxiety behaviour is consistent with previous employment of this paradigm (Vyas et al., 2002); however, it should be noted that this paradigm has previously been demonstrated to also elicit anxiolytic-like effects (d'Aquila et al., 1994). Clearly, the paradigm induced a prolonged stress response as indicated by the induction of adrenal hypertrophy. This suggests that the enhanced anxiety response elicited by the low dose of HU-210 was the result of a stress-induced increase in $CB_1$ receptor activity or a sensitization of one of its downstream mediators.

Previous research in our laboratory has demonstrated that this stress paradigm does not upregulate the $CB_1$ receptor in the limbic forebrain (Hill et al., 2003), the neuroanatomical region believed to mediate behavioural responses in the elevated plus maze. This suggests that the sensitization of anxiogenesis induced by a low dose of HU-210 may be attributed to sensitization in a system downstream of the $CB_1$ receptor. With regards to anxiety-like behaviour, much research suggests that differential cannabinoid-opioid interactions mediate the bidirectional effects of cannabinoids on anxiety (Berrendero and Maldonado, 2002; Marin et al., 2003). For example, it has been found that blockade of the μ and Δ-opioid receptors, but not the κ-opioid, can attenuate the anxiolytic effect elicited by low doses of delta-9-tetrahydrocannabinol (THC; Berrendero and Maldonado, 2002). Additionally, it has also been demonstrated that the anxiogenic effects elicited by high doses of cannabinoid administration can be attenuated by blockade of the κ-opioid receptor, but not by blockade of the μ or Δ-opioid receptors (Marin et al., 2003). Further, it has also been found that in mice that are deficient in dynorphin, the endogenous ligand for the κ-opioid receptor, there is an absence of aversive responses to high doses of THC (Zimmer et al., 2001). These findings suggest that cannabinoid induced activation of μ or Δ-opioid receptors may mediate the anxiolytic response elicited by low doses of cannabinoid agonists, whereas cannabinoid induced activation of dynorphin release and κ-opioid receptor activation may mediate the anxiogenic response seen after high doses. Thus, a sensitized anxiogenic response to a low dose of $CB_1$ receptor agonist could be the result of either a stress-induced decrement in μ or Δ-opioid receptor functioning, or an enhanced dynorphin or κ-opioid receptor response. Given that the anxiogenic response of cannabinoids appears to be mediated by κ-opioid receptor activation (Marin et al., 2003), it is tempting to predict that this effect is mediated by a stress-induced potentiation of this system. It has recently been shown that prolonged swim stress induces an enhancement of κ-opioid receptor functioning and that some of the aversive responses to stress are mediated by κ-opioid receptor activation through dynorphin release (McLaughlin et al., 2003). It is possible that the stress paradigm used here induced an increase in dynorphin production or κ-opioid receptor sensitivity. Thus, this change in opioid functioning would not have been apparent until it was initiated by cannabinoid receptor activation, and elicited an enhanced dysphoric response to a low dose of HU-210.

In conclusion, we have documented that exposure to prolonged stress enhances the anxiogenic response elicited by acute administration of a $CB_1$ receptor agonist at a low dose. These findings are reminiscent of clinical research, where humans suffering from chronic exposure to stress or stress-related disorders such as depression, may have an enhanced likelihood of an aversive response to cannabis consumption (Ablon and Goodwin, 1974).

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