Delta(9)-tetrahydrocannabinol Enhances an Increase of Plasma Corticosterone Levels Induced by Forced Swim-Stress

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The present study was designed to determine the effect of delta(9)-tetrahydrocannabinol (THC) on susceptibility to stress. We reported that THC significantly prolonged the immobility time during the forced swim-stress. The selective cannabinoid CB1 receptor antagonist O-2050 significantly reduced the enhancement of immobility by THC. We investigated the effect of THC on levels of stress hormone corticosterone under non-stress and forced swim-stress conditions. THC did not affect plasma corticosterone levels under non-stress conditions. However, THC, together with forced swim-stress, significantly increased plasma corticosterone levels. This effect was inhibited by O-2050. This evidence suggests that THC, under stressful conditions, enhances the susceptibility of the hypothalamus-pituitary-adrenal-axis to stress via the CB1 receptor, thereby increasing the risk of depression.

Key words delta(9)-tetrahydrocannabinol; corticosterone; forced swim-stress

Exposure to physically or emotionally aversive stimuli activates multiple stress systems, such as the adrenergic adrenal medulla system and the hypothalamus-pituitary-adrenal (HPA)-axis, which regulates glucocorticoid function.1) The HPA-axis is a key system in the maintenance of an organism’s homeostasis, a dynamic balance that is constantly challenged by internal and external stressors. However, an altered HPA-axis activity is associated with depression, and elevated plasma levels of cortisol, a glucocorticoid, and impaired glucocorticoid receptor negative feedback of the HPA-axis are exhibited in depressive disorder.2,3)

Cannabis is the most widely used illicit substance with for example, 40% of all Americans over the age of 12 years having used it at least once.4) Cannabis abuse is a risk factor for depression.5) Interestingly, the density of the cannabinoid CB1 receptor is increased in the prefrontal cortex of depressive symptoms.6)

Recent evidence supports a role for the cannabinoid system as a modulator of the HPA-axis.7) The endocannabinoids and the cannabinoid CB1 receptor are widely distributed in brain areas such as the hypothalamus, prefrontal cortex and hippocampus, areas that regulate the HPA-axis.8,9) Indeed, Di et al. reveals that endocannabinoids are involved in mediating the negative fast-feedback of the HPA-axis.10) Moreover, intracerebroventricular administration of delta(9)-tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana, increases plasma corticosterone, a glucocorticoid, levels.11) However, although cannabinoids play a role in the response to stress, there are no reports regarding the role of the cannabinoid system under conditions of stress. Cannabinoids might differentially affect HPA-axis activity, depending on the environmental context. Therefore, we investigated the effects of THC on plasma corticosterone levels under non-stress and forced swim-stress conditions.

MATERIALS AND METHODS

Animals Male ddY mice (Kyudo, Saga, Japan), aged 6 weeks, were housed in groups of seven in a temperature-controlled room (23 ± 2 °C) on a 12 h light–dark cycle (lights on 07:00—19:00), with food (CE-2; Clea Japan, Tokyo, Japan) and water available ad libitum. All procedures regarding animal care and use were performed in compliance with the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University.

Drugs THC was isolated from cannabis. THC and O-2050 (Tocris bioscience) were suspended in 1% Tween 80. All drugs were administered intraperitoneally (i.p.) 60 min prior to testing.

Forced Swim Test The forced swim procedure was carried out following a modified version of conditions proposed by Porsolt et al. as a suitable model for screening potential antidepressive agents.12) Briefly, animals were placed in individual plastic cylinders (diameter 11 cm; height 18 cm) filled with 10 cm of water (25 ± 1 °C) for 15 min and then removed and dried. Twenty-four hours later, a magnet was attached to one of their forelimbs, and they were placed back into the cylinders. We recorded the movement of the forelimb that had put up the magnet using the MicroAct Scratching Test (version 1.03; Neuroscience Inc., Tokyo, Japan) during a 5 min observation period (test session). We evaluated time that the forelimb did not move as immobility time.

Plasma Corticosterone Measurement Plasma corticosterone levels were measured between 12:00 and 14:00. Trunk blood was collected into micro tubes following decapitation, which was rapidly performed after forced swim-stress. After centrifugation for 15 min at 1500rpm at 4 °C, plasma samples were stored at −20 °C. Measurement of plasma corticosterone levels was performed using an enzyme immunoassay kit (Assay Designs, Inc., U.S.A.).

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Statistics The forced swim data and the group 2 of plasma corticosterone levels were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post-hoc test. The group 1 of plasma corticosterone levels were analyzed using the Student's t-test. The criterion for statistical significance was considered to be $p < 0.05$. The results were expressed as means±S.E.M.

RESULTS

THC dose-dependently prolonged the immobility time during the forced swim-stress. At doses of 2 and 6 mg/kg, THC significantly prolonged the immobility time (vehicle: 240.2±6.4 s, THC 1 mg/kg: 253.7±13.6 s, THC 2 mg/kg: 275.2±4.8 s, THC 6 mg/kg: 298.0±2.8 s, $F_{3,18} = 9.5$, $p < 0.01$, Fig. 1a). The selective cannabinoid CB1 receptor antagonist O-2050 at dose of 10 mg/kg significantly reduced the enhancement of immobility time by THC (6 mg/kg) (vehicle: 242.5±6.9 s, THC 6 mg/kg: 294.0±2.5 s, THC 6 mg/kg-O2050 1 mg/kg: 280.6±3.8 s, THC 6 mg/kg-O2050 10 mg/kg: 269.9±8.0 s, $F_{3,18} = 16.0$, $p < 0.01$, Fig. 1b). THC had no effect on plasma corticosterone levels before exposure to forced swim-stress on day 2 (vehicle: 347.7±62.7 ng/ml, THC 6 mg/kg: 398.2±44.2 ng/ml, Fig. 2). However, THC (6 mg/kg), together with forced swim-stress on day 2, significantly increased plasma corticosterone levels. This effect was inhibited by O-2050 (10 mg/kg) (vehicle: 327.4±28.0 ng/ml, THC 6 mg/kg: 684.1±87.2 ng/ml, THC 6 mg/kg-O2050 10 mg/kg: 374.7±12.5 ng/ml, $F_{2,12} = 13.2$, $p < 0.01$, Fig. 2).

DISCUSSION

THC (2, 6 mg/kg) significantly prolonged the immobility time of mice during the forced swim test, a test with a high predictability of antidepressant efficacy in human depression.$^{12}$ O-2050 (10 mg/kg) was also able to reverse the enhancement of immobility by THC. These results indicate that this enhancement effect is mediated by cannabinoid CB1 receptor. We previously reported that THC at a dose of 6 mg/kg did not significantly affect locomotor activity, motor coordination in rota-rod test and catalepsy-like immobilization.$^{13,14}$ Therefore, the THC-induced enhancement of immobility is unlikely to have been caused by deficits in motor function.

Recently, it has been reported that depression is implicated in hyperactivity of the HPA-axis. Indeed, elevated plasma cortisol levels are exhibited in depressive disorder.$^{25}$ Moreover, corticosterone exposure elicits depressive-like behavior.$^{25}$ Therefore, we investigated the effects of THC on plasma corticosterone levels. Basal corticosterone levels were 118.4±35.1 ng/ml in intact male mice, which were 366.9±37.7 ng/ml after forced swim-stress for 15 min on day 1 (data not shown). THC had no effect on plasma corticosterone levels under non-stress conditions on day 2. However, THC together with forced swim-stress significantly increased plasma corticosterone levels. This increase of corticosterone concentration was antagonized by O-2050, suggesting that THC, via the CB1 receptor, potentiates sensitivity of the HPA-axis to stress, which is involved in depressive-like behavior during the forced swim-stress. It has been reported that unpredictable stress and glucocorticoid treatment decreases expression of the CB1 receptor in the hippocampus.$^{16,17}$ The effect of glucocorticoids on CB1 receptor expression is be mediated by the ability of glucocorticoids to negatively regulate transcription of the CB1 receptor.$^{18}$ Therefore, it is suggested that an abnormality of glucocorticoid system by the forced swim-stress induces alteration of the endocannabinoid system, and the administration of THC in the condition enhances the responses to CB1 receptors. This idea is supported by several studies demonstrating that adrenactomy or pharmacological disruption of glucocorticoid receptor activity result in a potentiation of the responses to cannabinoid-related drug administration.$^{19–21}$ However, Schramm-Sapyta et al. have demonstrated that THC increases plasma corticosterone levels without stress exposure.$^{22}$ We used mice, while Schramm-Sapyta et al. used rats. The synthetic cannabinoid agonist WIN 55,212-2
induces anxiolytic effect via GABAergic mechanisms in mice and anxiogenic effect via glutamatergic mechanisms in rats.\textsuperscript{23} Thus, this discrepancy might depend on species difference in the effects of THC on an activation of the HPA-axis.

It has been reported that WIN 55,212-2 is not able to modify basal adrenocorticotropic hormone (ACTH) levels, but simultaneous application of corticotropin-releasing hormone (CRH) and WIN results in a synergistic effect on ACTH secretion from the human pituitary gland, and this effect is abolished by the CB\textsubscript{1} antagonist SR141716.\textsuperscript{24} Furthermore, CRH mRNA in the paraventricular nucleus of the hypothalamus is increased by restraint stress.\textsuperscript{25} Taken together with these observations, the present results suggest that THC might act to enhance the susceptibility of the HPA-axis to stress via a synergistic action with stress-induced elevated CRH levels. However, the functional significance of CB\textsubscript{1} receptor in the HPA-axis remains to be elucidated. Further experiments will be needed to clarify the possible role of the cannabinoid system in the modulation of the HPA-axis.

In conclusion, the study presented here demonstrates for the first time that THC, acting synergistically with stress, increases plasma corticosterone levels. These findings suggest that, in cannabis users, the susceptibility of the HPA-axis to stress is enhanced in stressful situations, thereby increasing the risk of depression.

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REFERENCES