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Cannabinoid-induced upregulation of serotonin 2A receptors in the hypothalamic paraventricular nucleus and anxiety-like behaviors in rats☆

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HIGHLIGHTS

- Repeated CP55940 treatment enhances 5-HT_{2A}-mediated neuroendocrine responses.
- 5-HT_{2A} protein levels in PVN were increased after repeated CP55940 treatment.
- 5-HT_{2A} and Gαq mRNA levels in PVN were not modified by CP55940 treatment.
- CP55940 treatment increases anxiety-like behavior in rats.

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ABSTRACT

Recent behavioral reports suggest that repeated exposure to cannabis and synthetic cannabinoid agonists is linked with mental disorders associated with dysfunction of serotonin 2A (5-HT_{2A}) receptor neurotransmission such as anxiety and depression. Here, we studied the effect of a nonselective cannabinoid agonist, CP55940, on the activity of 5-HT_{2A} receptors in hypothalamic paraventricular nucleus (PVN). We detected that repeated exposure to CP55940 enhanced the prolactin and corticosterone neuroendocrine responses mediated by 5-HT_{2A} receptors and increased the membrane-associated levels of 5-HT_{2A} receptors in PVN. Importantly, we also detected increased anxiety-like behaviors in CP55940 treated rats compared to controls. The data presented here suggest that the mechanisms mediating the cannabinoid-induced upregulation of 5-HT_{2A} receptors would be brain-region specific, as we were unable to detect a CP55940-induced upregulation of 5-HT_{2A} mRNA. Our results might provide insight into the molecular mechanism by which repeated exposure to cannabinoids could be associated with the pathophysiology of neuropsychiatric disorders.

1. Introduction

A number of recent behavioral studies suggest that administration of Δ^9-tetrahydrocannabiniol (Δ^9-THC), the main psychoactive component of marijuana, or several synthetic cannabinoids can regulate the activity of serotonin 2A (5-HT_{2A}) receptors [11,24]. While acute cannabinoid administration reduces 5-HT_{2A} receptor-mediated behavioral responses [11]; repeated exposure to cannabinoids seems to be associated with increased behavioral responses to 5-HT_{2A} receptor agonists in adult rats [24]. Accordingly, we have recently reported that repeated exposure to cannabinoid agonists upregulates and increases the activity of 5-HT_{2A} receptors in rat prefrontal cortex (PFCx) and in two neuronal cell models [17–20].

Cannabinoids produce their responses by activating two cannabinoid receptors, CB_1 and CB_2 receptors, in the brain [8]. These receptors are expressed in different areas of the brain, including PVN, amygdala, cerebellum, hippocampus, and cortex [1,2,13,16,22]. CB_1 and CB_2 receptors couple to $G_{i/o}$ class of G-proteins and to the extracellular kinase (ERK) signaling pathway [4,8]. In our previous studies, we have reported that the cannabinoid-induced upregulation of 5-HT_{2A} receptors in two neuronal cell models is mediated by CB_2 receptors and ERK1/2 activation as it is inhibited in cells treated with CB_2, but not CB_1, shRNA lentiviral particles and by ERK1/2 inhibitors [17–19].

Activity of 5-HT_{2A} receptors in either PFCx or hypothalamic paraventricular nucleus (PVN) has been associated with several physiological functions and neuropsychiatric disorders such as stress response, anxiety and depression and schizophrenia [6,12].

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT_{2A}, serotonin 2A; Δ^9-THC, Δ^9-tetrahydrocannabinol; ERK, extracellular kinase; PVN, hypothalamic paraventricular nucleus; CP 55,940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; (−) DOI, [(−)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane HCl].

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Although the clinical manifestations of the cannabinoid-induced upregulation of 5-HT_{2A} receptors are currently under discussion, it has been suggested that repeated exposure to nonselective cannabinoid agonists might fasten the onset of the neuropsychiatric disorders described above [6,23,26,27,34]. Of note, recent preclinical studies indicated that chronic, but not acute, exposure to non-selective [30,31] or selective CB_2 receptor agonists induced anxiety-like behaviors in rodents [21].

Here, we investigated the effect of repeated exposure to CP55940 on the activity of 5-HT_{2A} receptors in the hypothalamic PVN. CP55940 is a nonselective cannabinoid agonist (CB_1 and CB_2 agonist, K_i: 0.58 nM and 0.68 nM for CB_1 and CB_2 receptors, respectively) [35]. We measured [(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl] (−)DOI, 5-$HT_{2A/AC}$ agonist, induced changes in neuroendocrine responses, corticosterone and prolactin plasma levels, as an index of activity of 5-HT_{2A} receptors in PVN. We have previously demonstrated that the neuroendocrine effects of (−)DOI are mediated exclusively by activation of 5-HT_{2A} receptors, but not 5-HT_{2C} receptors, in rat PVN [39,41]. Here we reported a cannabinoid agonist-induced upregulation of 5-HT_{2A} receptors in hypothalamic PVN and increased-anxiety like responses in rats treated with CP55940. We hypothesize that these studies will further our understanding of the neurobiological mechanisms associated with repeated cannabinoid exposure.

2. Materials and methods

2.1. Drugs

CP55940, a CB_1/CB_2 agonist, was purchased from Tocris (Ellisville, MO). A fresh CP55940 solution (0.05 mg/ml) was prepared in Tween-80/ethanol/saline (1:1:18) prior to each dosing. (−) DOI [(−)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane HCl] was purchased from Sigma–Aldrich Inc. (St. Louis, MO) and dissolved in 0.9% saline at one concentration (0.35 mg/kg, s.c.). All solutions were made fresh before administration and injected at a volume of 1 ml/kg.

2.2. Animal experimental protocol

Male Sprague-Dawley rats (225–275 g) were purchased from Harlan (Indianapolis, IN). The rats were housed two per cage in a temperature-, humidity-, and light-controlled room (12 h light/dark cycle, lights on 7:00 AM–19:00 PM). Food and water were available ad libitum. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the University of Kansas Institutional Animal Care and Use Committee (IACUC).

After arrival, the rats were allowed to acclimate to their environment for at least 7 days prior to the start of the treatment period. Eight rats were randomly assigned to each group. Cage-mates were assigned to the same treatment group. Rats were injected with either vehicle (1 ml/kg, i.p.) or CP55940 (0.05 mg/kg, i.p.) once a day for 7 days. Rats were sacrificed by decapitation 48 h after the last CP55940 injection. The rats were challenged with either saline (1 ml/kg) or (−)DOI (0.35 mg/kg) 30 min prior to sacrifice. Trunk blood was collected for hormone assays and brain tissues were rapidly obtained and frozen in dry ice.

2.3. Radioimmunoassay

Plasma prolactin and corticosterone concentrations were determined by radioimmunoassays as previously described [7].

2.4. Western blots

Membrane-associated proteins were isolated using the ProteoExtract™ Native Membrane Protein Extraction kit (Calbiochem, La Jolla, CA) according to manufacturer's instructions. Briefly, PVN tissue was homogenized in extraction buffer I containing protease inhibitor cocktail. Homogenates were incubated for 15 min at 4 °C under gentle agitation, centrifuged 16,000 × *g* for 15 mins at 4 °C, and then the supernatant containing the cytosolic fraction was collected. The pellet was incubated with extraction buffer II with protease inhibitor cocktail for 30 min at 4 °C under gentle agitation and centrifuged 16,000 × *g* for 15 min at 4 °C to isolate the membrane fraction contained in the supernatant. Expression of membrane-associated 5-HT_{2A} receptors in PVN was determined by Western blot as previously described [7]. Films were analyzed densitometrically using Scion Image software (Scion Corporation, Frederick, MD, USA) as previously described [7]. Each sample was measured on three independent gels. All samples were standardized to controls and normalized to their respective actin levels.

2.5. Quantitative real-time PCR

These reactions were prepared using QuantiFast SYBR Green PCR Kit (Qiagen, Valencia, CA), the ABI 7500 fast real time PCR system (Applied Biosystems, Foster City, CA) and then data was analyzed using the comparative cycle threshold (Ct) method as described [36]. The primers used in this manuscript were: 5-HT_{2A} (F:5′-AACGGTCCATCCACAGAG-3′,R:5′-AACAGGAAGAACACGATGC-3′), $G\alpha_q$ (F:5′-AGTTCGAGTCCCCACCACAG-3′,R:5′-CCTCCTACATCGACCATTCTGAA-3′), and GAPDH (F:5′-TGGAGTCTACTGGCGTCTTCAC-3′,R:5′-GGCATGGACTGTGGTCATGA-3′). These primers have been previously validated [3,25,29].

2.6. Behavioral tests

We used a separate group of rats to measure anxiety-like behaviors in rats injected with either vehicle (1 ml/kg, i.p.) or CP55940 (0.05 mg/kg, i.p.) once a day for 7 days. 48 h after the last CP55940 injection, anxiety-like behaviors and locomotor activity were assessed in an elevated plus maze (Med Associates, St. Albans, VE) as previously described [32,37].

2.7. Statistics

All data are expressed as the mean ± SEM, where *n* indicates the number of rats per group. Data was analyzed by an unpaired Student's *t*-test or ANOVA (Newman–Keuls post hoc test). GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD, USA) was used for statistical analyses.

3. Results

We first examined the effect of repeated administration of CP55940, a CB_1/CB_2 receptor agonist [4], on the activity and expression of 5-HT_{2A} receptors in PVN. Rats were treated with CP55940 once a day for 7 days and then were challenged with (−)DOI (5-$HT_{2A/2C}$ receptor agonist) 30 min prior to sacrifice. We used this challenge to measure activity of 5-HT_{2A} receptors in PVN because the neuroendocrine effects of (−)DOI, are mediated exclusively by activation of 5-HT_{2A} receptors, but not 5-HT_{2C} receptors, in PVN [7]. In (−)DOI-challenged rats, we found significant ($p < 0.05$) increases in 5-HT_{2A} receptor-mediated prolactin (Fig. 1A) and corticosterone (Fig. 1B) plasma levels. The levels of prolactin