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Delta FosB and AP-1-mediated transcription modulate cannabinoid CB_1 receptor signaling and desensitization in striatal and limbic brain regions

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ABSTRACT

Repeated Δ^9-tetrahydrocannabinol (THC) administration produces cannabinoid type 1 receptor (CB_1R) desensitization and downregulation, as well as tolerance to its in vivo pharmacological effects. However, the magnitude of CB_1R desensitization varies by brain region, with CB_1Rs in the striatum and its output nuclei undergoing less desensitization than other regions. A growing body of data indicates that regional differences in CB_1R desensitization are produced, in part, by THC-mediated induction of the stable transcription factor, ΔFosB, and subsequent regulation of CB_1Rs. The purpose of the present study was to determine whether THC-mediated induction of ΔFosB in the striatum inhibits CB_1R desensitization in the striatum and output nuclei. This hypothesis was tested using bitransgenic mice with inducible expression of ΔFosB or ΔcJun, a dominant negative inhibitor of AP-1-mediated transcription, in specific forebrain regions. Mice were treated repeatedly with escalating doses of THC or vehicle for 6.5 days, and CB_1R-mediated G-protein activation was assessed using CP55,940-stimulated [^{35}S]GTPγS autoradiography. Overexpression of ΔFosB in striatal dopamine type 1 receptor-containing (D_1R) medium spiny neurons (MSNs) attenuated CB_1R desensitization in the substantia nigra, ventral tegmental area (VTA) and amygdala. Expression of ΔcJun in striatal D_1R- and dopamine type 2 receptor (D_2R)-containing MSNs enhanced CB_1R desensitization in the caudate-putamen and attenuated desensitization in the hippocampus and VTA. THC-mediated in vivo pharmacological effects were then assessed in ΔcJun-expressing mice. Tolerance to THC-mediated hypomotility was enhanced in ΔcJun-expressing mice. These data reveal that ΔFosB and possibly other AP-1 binding proteins regulate CB_1R signaling and adaptation in the striatum and limbic system.

1. Introduction

Δ^9-Tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana, elicits behavioral effects by activating cannabinoid CB_1 receptors (CB_1Rs) in the central nervous system [1,2]. Repeated THC administration produces CB_1R desensitization, measured as a reduction in receptor-mediated G-protein or effector activity [3], which occurs concomitantly with tolerance to THC-mediated in vivo effects [4]. CB_1R desensitization varies in magnitude by brain region depending on the dose and duration of repeated cannabinoid administration. Moreover, the brain regional profile of desensitization corresponds with the development of tolerance to specific cannabinoid-mediated responses [3]. For example, CB_1R desensitization in the dorsal striatum and its output nuclei (i.e., globus pallidus, substantia nigra) is generally lower in

Abbreviations: AMYG, amygdala; BSA, bovine serum albumin; CaMKIIα, calcium/calmodulin-dependent protein kinase II; CB_1R, cannabinoid type 1 receptor; CBLM, cerebellum; CP55,940, (−)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol; CPU, caudate-putamen; D_1R, dopamine type 1 receptor; D_2R, dopamine type 2 receptor; ERK, extracellular signal-regulated kinase; GP, globus pallidus; GDP, guanosine diphosphate; GTPγS, guanosine 5′-(gamma-thio)triphosphate; HIP, hippocampus; MSN, medium spiny neuron; NAC, nucleus accumbens; PFC, prefrontal cortex; SN, substantia nigra; THC, Δ^9-tetrahydrocannabinol; VTA, ventral tegmental area.

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magnitude and requires higher drug doses and/or longer treatment duration than other brain regions [3,5,6]. Studies using post-mortem autoradiography or in vivo imaging in brains from marijuana users compared to non-users have also revealed a smaller decrease in CB_1R levels in the caudate-putamen compared to other regions, including hippocampus [7,8]. These findings are consistent with reports in human marijuana users that showed greater tolerance to the memory impairing effects of THC, which are hippocampal-dependent, than to motor impairment and subjective "high", which involve striatal circuits [9]. These data suggest the potential functional and translational relevance of regional differences in CB_1R adaptation, but the regulatory mechanisms that underlie these region-specific effects are not known.

We have proposed that regional differences in the interaction of CB_1Rs with specific signaling and regulatory proteins contribute to region-specific differences in CB_1R adaptation [3,10,11] and recently suggested that the induction of transcription factors following repeated THC administration plays a modulatory role in CB_1R desensitization [12]. This idea was based, in part, on the demonstration that an inverse regional relationship exists between THC-mediated CB_1R desensitization and induction of ΔFosB [13]. For example, repeated THC treatment induces ΔFosB expression in the striatum, a region that generally shows lower CB_1R desensitization. ΔFosB belongs to the Fos family of transcription factors, which dimerize with Jun proteins to produce an AP-1 complex that regulates the transcription of target genes [14,15]. ΔFosB, a truncated splice variant of FosB, is stable and accumulates with repeated drug administration [16]. Inducible transgenic overexpression of ΔFosB in dopamine type 1 receptor (D_1R) positive striatal medium spiny neurons (MSNs) showed enhanced rewarding effects of drugs of abuse and natural rewards [17,18]. In contrast, expression of ΔcJun, a dominant negative inhibitor of ΔFosB-mediated transcription, in both D_1R and dopamine type 2 receptor (D_2R) positive MSNs reduced cocaine- and morphine- induced condition place preference [19,20]. ΔFosB produces functional effects by regulating the expression of target genes that include certain receptors and signaling proteins [21], which can affect receptor-mediated signaling. For example, inducible transgenic overexpression of ΔFosB in the nucleus accumbens enhanced mu opioid, but not CB_1, receptor-mediated G-protein activity and inhibition of adenylyl cyclase [22]. Although ΔFosB might not play a pivotal role in CB_1R signaling acutely, it might modulate CB_1R desensitization. The current study tested the hypothesis that repeated THC administration to transgenic mice that inducibly overexpress ΔFosB or its transcriptional inhibitor ΔcJun in the forebrain will display a difference in CB_1R desensitization, as assessed by CP55,940-stimulated [^{35}S]GTPγS binding. Additionally, tolerance to common pharmacological effects (hypomotility, antinociception, catalepsy, and hypothermia) of THC was quantified in transgenic mice treated repeatedly with vehicle or THC.

2. Materials and methods

2.1. Materials

THC, [(−)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol] (CP55,940) and SR141716A were provided by the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). [^{35}S]Guanosine 5′-(gamma-thio)triphosphate (GTPγS; 1250 Ci/mmol), [^{3}H]SR141716A (54 Ci/mmol) and Kodak Biomax MR film were purchased from PerkinElmer Life Sciences (Boston, MA). Bovine serum albumin (BSA), guanosine diphosphate (GDP) and doxycycline were purchased from Sigma-Aldrich (St. Louis, MO). ScintiSafe Econo 1 scintillation fluid was obtained from Research Products International (Mount Prospect, IL). Whatman glass fiber filters were purchased from Brandel (Gaithersburg, MD). All other reagent grade chemicals were obtained from Sigma Chemical Co. or Fisher Scientific.

2.2. Subjects and drug treatments

Subjects were male, bitransgenic *NSE-tTA* × *TetOp-ΔFosB* mice and *NSE-tTA* × *TetOp-FLAG-Δc-Jun* mice with brain-region specific, tetracycline-regulated inducible expression of either ΔFosB or ΔcJun, respectively [19,23]. ΔFosB or ΔcJun expression is controlled by adding doxycycline to the drinking water, which prevents ΔFosB/ΔcJun expression. Therefore, omission of doxycycline from the drinking water allows ΔFosB/ΔcJun to be expressed. In mice that overexpress ΔFosB, expression is found in D_1R-positive MSNs in the caudate-putamen and nucleus accumbens, deep layers of cerebral cortex and hippocampus [23]. In mice that overexpress ΔcJun, expression occurs in both D_1R and D_2R positive MSNs in the caudate-putamen and nucleus accumbens, parietal cortex and hippocampus [19]. ΔcJun is a dominant negative functional inhibitor of AP-1-mediated transcription, thus this model provides a strategy to block the effects of THC-induced ΔFosB expression. Mice were housed four to six per cage and maintained on a 12-h light:12-h dark cycle in a temperature-controlled environment (20–22 °C) with food and water available ad libitum. Mice were maintained on drinking water that contained doxycycline (100 μg/ml) throughout gestation and were either taken off doxycycline for 8 weeks prior to experiments to induce expression of ΔFosB or ΔcJun (ΔFosB-ON, ΔcJun-ON) or maintained on doxycycline (control; ΔFosB-OFF, ΔcJun-OFF). After 8 weeks with/without doxycycline, mice were injected subcutaneously twice daily (08:00 and 16:00) for 6 days with vehicle (1:1:18 solution of ethanol, emulphor and saline) or THC doses that increased every 2 days (10–30–60 mg/kg, injection) [5]. On day 7, mice for autoradiographic studies received only the morning injection of THC or vehicle, and 24 h later brains were collected for CP55,940-stimulated [^{35}S]GTPγS binding. This THC treatment regimen was employed because it produces CB_1R desensitization throughout the brain, including the striatum, and therefore should reveal whether ΔFosB expression alters CB_1R desensitization. All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Virginia Commonwealth University in accordance with the National Institutes of Health guide for the care and use of Laboratory animals 8th edition.

2.3. Agonist-stimulated [^{35}S]GTPγS autoradiography

Assays were conducted as previously published [10,24]. Coronal brain sections (20 μm) were cut on a cryostat maintained at −20 °C, thaw-mounted onto gelatin-coated slides and stored desiccated at 4 °C overnight. Sections were collected at levels that included (1) prefrontal cortex, (2) nucleus accumbens, (3) caudate-putamen, (4) globus pallidus, (5) hippocampus and amygdala (including central, basolateral and basomedial nuclei), (6) VTA, (7) substantia nigra, and (8) cerebellum. Slides were stored desiccated at −80 °C until use. On the day of the assay, slides were brought to room temperature, rinsed in 50 mM Tris–HCl buffer (pH 7.4) with 3 mM $MgCl_2$, 0.2 mM EGTA and 100 mM NaCl (TME Buffer) for 10 min at 25 °C. Slides were transferred to TME Buffer + 0.5% BSA with 2 mM GDP and 10 mU/ml adenosine deaminase for 15 min at 25 °C. Slides were then incubated in TME Buffer + 0.5% BSA containing 0.04 nM [^{35}S]GTPγS, 2 mM GDP, 10 mU/ml adenosine deaminase and 3 μM CP55,940 or vehicle (ethanol) for 2 h at 25 °C. CP55,940 was selected because it does not stimulate [^{35}S]GTPγS binding in brain sections from CB_1R knockout mice [25], thereby