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SCIENCE DIRECT
Brain Research 1048 (2005) 41 – 47
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Effect of Δ^9-tetrahydrocannabinol on phosphorylated CREB in rat cerebellum: An immunohistochemical study

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Accepted 13 April 2005
Available online 23 May 2005

Dedicated to the memory of Anna Porcella, a wonderful friend and scientist who died in September 2003. Her work on CREB and cannabinoids was the basis of our study.

Abstract

Several converging lines of evidence indicate that drugs of abuse may exert their long-term effects on the central nervous system by modulating signaling pathways controlling gene expression. Cannabinoids produce, beside locomotor effects, cognitive impairment through central CB1 cannabinoid receptors. Data clearly indicate that the cerebellum, an area enriched with CB1 receptors, has a role not only in motor function but also in cognition. This immunohistochemical study examines the effect of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of marijuana, on the levels of phosphorylated CREB (p-CREB) in the rat cerebellum. Acute treatments with Δ^9-THC at doses of 5 or 10 mg/kg induced a significant increase of p-CREB in the granule cell layer of the cerebellum, an effect blocked by the CB1 receptor antagonist SR 141716A. Following chronic Δ^9-THC administration (10 mg/kg/day for 4 weeks), the density of p-CREB was markedly attenuated compared to controls, and this attenuation persisted 3 weeks after withdrawal from Δ^9-THC.

These data provide evidence for the involvement of cerebellar granule cells in the adaptive changes occurring during acute and chronic Δ^9-THC exposure. This might be a mechanism by which Δ^9-THC interferes with motor and cognitive functions.

Theme: Neural basis of behavior
Topic: Drugs of abuse: opioids and others

Keywords: Cannabinoids; CREB; Cerebellum; Immunohistochemistry

1. Introduction

Cannabinoid receptors CB1 are expressed at a very high density in the cerebellum, an area of the brain implicated in motor coordination, so it is not surprising that in humans cannabinoids such as Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of marijuana, have complex effects on psychomotor function. In mice, direct injection of synthetic cannabinoids into the cerebellum produces motor impairments in the rotorod test, and these deficits are no longer seen in animals that have cerebellar injections of an antisense oligonucleotide directed to a sequence coding for CB1 receptors [11]. Although it has long been known in clinical neurology as much as in experimental neuroscience that the cerebellum is essential for the co-ordination of movement, a growing body of evidence has also implicated the cerebellum in diverse higher cognitive functions. For example, patients with cerebellar diseases have impaired spatial cognition, executive dysfunctions with difficulties in planning, abstraction and working

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doi:10.1016/j.brainres.2005.04.053

memory [29]. These observations raise the possibility that the cerebellar mechanisms implicated in learning and memory might also be relevant in the mechanism of action of cannabinoids. The intake of marijuana induces, beside locomotor effects such as hypolocomotion, ataxia and catalepsy, clear cognitive impairments [32]. Prolonged marijuana usage disrupts short-term memory, working memory, attentional skills and memory retrieval [4]. Administration of cannabis extracts also causes long-lasting memory deficits in rodents, and similar deficits are produced by either Δ^9-THC, endogenous cannabinoids or different synthetic CB_1 cannabinoid receptor agonists [6,18,19,21,31].

Several of the molecular and cellular adaptations involved in addiction are believed to be also implicated in learning and memory. Of particular interest is the activation of the cAMP pathway and CREB-mediated transcription which have been related to learning and long-term potentiation of synaptic transmission [35]. Numerous CNS processes, including neurotransmitter synthesis, gene expression and cellular proliferation, are controlled by neurotransmitters acting through second messenger systems that phosphorylate the transcription factor CREB (cyclic AMP response element-binding protein). CREB is an ubiquitously expressed protein regulated by several intracellular pathways that binds to specific DNA sequences (named CREs or cAMP-response elements) in the regulatory regions of target genes [24]. The transcriptional activity of these dimers is stimulated upon phosphorylation of CREB at Ser133 by several protein kinases, including protein kinase A, Ca^{2+}/calmodulin-kinase II and IV and several kinases in the mitogen-activated protein-kinase cascade (MAPK). Thus, CREB represents a site of convergence where diverse signaling pathways and their associated stimuli produce plasticity by altering gene expression [30].

Cannabinoids may exert their effects on brain and behavior by modulating signaling pathways controlling gene expression. Accordingly, studies have demonstrated that Δ^9-THC induces the expression of the immediate-early gene c-*fos* [20]. Furthermore, acute administration of Δ^9-THC in rats induces a progressive and transient activation of extracellular signal-regulated kinase (ERK) in the dorsal striatum, nucleus accumbens and hippocampus [10,33]. It has recently been reported that acute Δ^9-THC increases components of the ERK pathway (ERK, p-CREB and c-*fos*) in the rat cerebellum, while repeated treatment with Δ^9-THC prevents this acute effect of Δ^9-THC [28].

Given that learning, memory and drug addiction share some intracellular signaling cascade events dependent on the activation of CREB [25] and the relationships existing between cognitive deficits, the cerebellum and the adverse effects of cannabinoids, it was important to better understand the effect of Δ^9-THC on CREB in the rat cerebellum. It was still unknown where in the cerebellum an acute Δ^9-THC-induced response might be displayed. Furthermore, it was crucial to know whether chronic Δ^9-THC would alter levels of activated CREB a day or several weeks after the last administration of Δ^9-THC. To this aim, we performed an immunohistochemical study on the cerebellum of acutely or chronically treated rats using an antibody raised against phosphorylated CREB (p-CREB), the activated form that binds to DNA.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley albino rats (Charles River, Italy) were housed in groups of 5 in standard plastic cages with water and food ad libitum. The animal facility was under a 12:12 h light–dark cycle, constant temperature of 22 ± 2 °C and relative humidity of 60%. All experimental protocols were performed in strict accordance with the E.C. regulation for care and use of experimental animals (CEE No. 86/609).

The appropriate concentration of Δ^9-THC (purchased as a 10 mg/ml in ethanol solution, from Sigma, St. Louis, Missouri, USA) was prepared by evaporating the alcohol with nitrogen and emulsifying the residue with Cremophor, ethanol and saline (1:1:18). For the acute treatment, rats received vehicle (n = 10) or Δ^9-THC at the doses of 2.5 (n = 10), 5 (n = 10) and 10 mg/kg i.p. (n = 10) and were sacrificed 90 min after for immunohistochemistry. A separate group of rats (n = 5) received an acute injection of SR 141716A (1 mg/kg) 15 min before the treatment with Δ^9-THC or its vehicle. For chronic treatments, rats (n = 12) were injected intraperitoneally with Δ^9-THC once a day for 4 weeks at a dose of 10 mg/kg and were sacrificed 12 h (n = 6) or 3 weeks after (n = 6). Control animals (n = 6) were given vehicle for the same time.

Δ^9-THC-treated animals presented typical behavioral cannabinoid effects such as a reduction in spontaneous locomotor activity.

2.2. Immunohistochemistry

Rats were perfused transcardially 90 min after Δ^9-THC treatment with 4% paraformaldehyde in 0.2 M phosphate buffer (PB). The brains were subsequently cryoprotected overnight with a solution of 30% sucrose in 0.1 M PB at 4 °C. Alternate sagittal sections of 40 μm were cut at sledge (Microm HM 400 R). Adjacent sections were processed for Nissl staining (cresyl violet from Sigma) or p-CREB immunohistochemistry. For the immunohistochemistry, controls were performed by subtracting the primary antibody in the procedure. The staining was performed as previously reported [8]: after rinsing in phosphate-buffered saline with 0.2% Triton X-100 (PBS + T), sections were incubated with 0.3% of H_2O_2 in PBS and, after extensive washing, with a blocking solution containing 1% BSA and 20% normal goat serum in PBS + T to reduce background.