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Short communication

Δ^9-Tetrahydrocannabinol increases proopiomelanocortin gene expression in the arcuate nucleus of the rat hypothalamus

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Abstract

Δ^9-Tetrahydrocannabinol, the main psychoactive component of cannabis, produces a large spectrum of pharmacological effects, many of which have been linked to interaction with the opioid system. The aim of this study was to examine the effects of Δ^9-tetrahydrocannabinol on proopiomelanocortin (POMC) gene expression in the arcuate nucleus of the hypothalamus and anterior lobe of the pituitary. We report, for the first time, that a 5-day treatment with Δ^9-tetrahydrocannabinol (5 mg/kg per day, i.p.) increased (38%) POMC mRNA levels in the arcuate nucleus of the hypothalamus but was without effect in the anterior lobe of the pituitary. These data indicate that Δ^9-tetrahydrocannabinol stimulates opioid gene expression and regulates distinctively POMC in the hypothalamus and the anterior lobe of the pituitary in the rat.

Keywords: Δ^9-Tetrahydrocannabinol; Opioid; Proopiomelanocortin; mRNA; Arcuate nucleus

1. Introduction

Δ^9-Tetrahydrocannabinol is the major psychoactive component of the preparations of *Cannabis sativa* (marihuana, hashish, bhang), responsible of its pharmacological actions in the central nervous system (CNS) including hypothermia, depressed motor activity, hypotension, inhibition of intestinal motility and antinociception (reviewed in Dewey, 1986). Several lines of evidence suggest that cannabinoids interact with the opioid system: (1) Δ^9-tetrahydrocannabinol is known to inhibit, in a non-competitive manner, the binding of μ and δ, but not κ-opioid receptor ligands to rat brain membranes (Vaysse et al., 1987); (2) rodents chronically treated with crude hashish extracts or purified Δ^9-tetrahydrocannabinol develop an opioid-like withdrawal syndrome after acute naloxone administration (Kaymakcalan et al., 1977; Vela et al., 1995); and (3) Δ^9-tetrahydrocannabinol-induced analgesia can be blocked by previous administration of μ- or κ-opioid selective antagonists or antibodies for dynorphin$_{1-8}$ (Reche et al., 1996).

β-Endorphin precursor proopiomelanocortin (POMC) concentrating neurons are located in the arcuate nucleus of the hypothalamus. These neurons project to numerous brain regions including hypothalamus, limbic system, midbrain periaqueductal gray and spinal cord. Such a complex pattern of connectivity makes the arcuate nucleus an important nucleus capable to integrate emotional and sensory information, particularly pain, with endocrine function (Chronwall, 1985). POMC mRNA-derived peptides have also been detected in the anterior lobe of the pituitary gland. In this tissue, the post-translational proteolytic processing of POMC produces predominantly β-lipotropin and adrenocorticotropin.

Due to the extended use of marihuana in the population, it is pertinent to further explore the effects of cannabinoids by examining whether this substance of abuse may alter the expression of opioid genes involved in the regulation of hormones and pain. To this aim, we have investigated, by quantitative in situ hybridization histochemistry, the effects of 5 days treatment with Δ^9-tetrahydrocannabinol on POMC gene expression in the arcuate nucleus of the hypothalamus and the anterior lobe of the pituitary gland.

2. Material and methods

Adult male Sprague-Dawley rats, weighing 200–225 g, were obtained from Interfauna Iberica Laboratories (San

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Feliu de Codines, Barcelona, Spain) and maintained under conditions of controlled temperature (23 ± 1°C) and lighting (lights on 08:00–20:00 h), with food and water provided ad libitum.

Δ^9-Tetrahydrocannabinol (generously provided by the National Institute of Drug Abuse, NIDA, Baltimore, MD, USA) was dissolved in saline/ethanol/cremophor (18:1:1) and administered (5 mg/kg, i.p.; 1 ml/kg) daily at 10:00 a.m. for 5 days. Five hours after the last injection rats were killed by decapitation and brains and pituitaries were quickly removed and frozen over solid CO_2. Twelve μm coronal brain and pituitary sections (3 slides/area; 2 sections/slide), were mounted onto gelatin-coated slides and stored at −80°C until the day of the assay.

In situ hybridization histochemistry was performed as described previously (Young et al., 1986) using a synthetic 38-base oligonucleotide probe complementary to the sequence 96–134 of the rat POMC gene (obtained from the Advanced Biotechnology Center, Charing Cross and Westminster Medical School, London, UK). The oligonucleotide probe was labeled using terminal deoxytransferase (Boehringer-Mannheim, Madrid, Spain) to add a ^{35}S-labeled deoxyATP (1000 Ci mmol^{-1}; Amersham, Madrid, Spain) tail to the 3′ end of the probe. The probe (in 45 μl of hybridization buffer) was applied to each section and left overnight at 37°C for the hybridization. Following hybridization, sections were washed 4 times for 15 min each in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2 (1× saline sodium citrate, SSC) at 55°C, followed by two 30 min washes in 1× SSC at room temperature, one brief water dip and blown dry with air. The dried slides were apposed to Hyperfilm β-max (Amersham, Madrid, Spain) 18 h for anterior lobe of the pituitary and 20 days for the arcuate nucleus of the hypothalamus. Autoradiograms were analyzed with a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). Optical densities were calculated from the uncalibrated mode expressed in grey scale values. Results were presented considering mean control values as 100%.

Statistical analysis was performed using Student's two-tailed, paired *t*-test. Differences were considered significant if the probability of error was less than 5%.

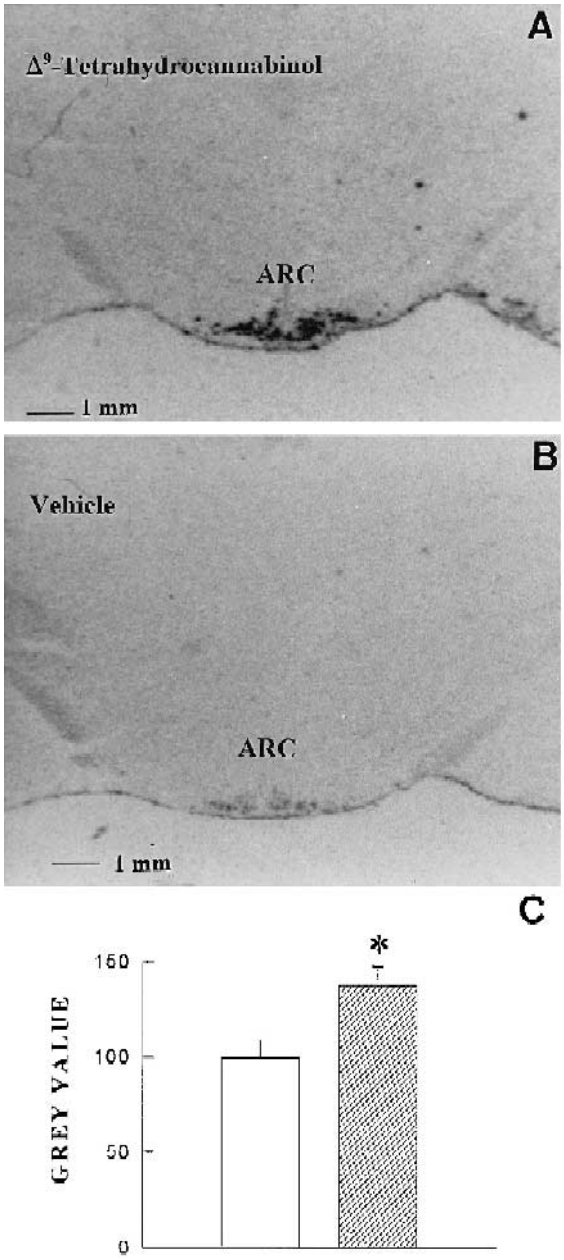

Fig. 1. Effects of Δ^9-tetrahydrocannabinol and vehicle on POMC mRNA levels in the arcuate nucleus of the hypothalamus. Autoradiograms of coronal brain sections of the arcuate nucleus of the hypothalamus of Δ^9-tetrahydrocannabinol- (panel A) and vehicle- (panel B) treated rats. Levels of POMC mRNA were measured in the arcuate nucleus of rats 5 days after i.p. administration of Δ^9-tetrahydrocannabinol (hatched column, 5 mg/kg per day) or vehicle (open column, saline/ethanol/cremophor; 18:1:1, 1 ml/kg). In panel C, values represent the mean ± S.E.M of POMC mRNA levels (expressed as % of grey level) in 6 rats. * Values from Δ^9-tetrahydrocannabinol-treated rats that are significantly different from vehicle-treated rats (Student's *t*-test, $P < 0.05$).

3. Results

The effects of Δ^9-tetrahydrocannabinol on POMC mRNA levels in the arcuate nucleus are shown in Fig. 1. The distribution of POMC gene expression in the arcuate nucleus of the hypothalamus is similar to that reported by others (Chronwall, 1985). The intensity of the hybridization signal is clearly more intense in the arcuate nucleus of the animals treated with Δ^9-tetrahydrocannabinol when compared with the vehicle-treated group. Indeed, the mean (6 serial sections per animal) optical density significantly increased by 38% in the group of animals treated with repeated injections of Δ^9-tetrahydrocannabinol. By contrast, POMC mRNA levels were not altered in the anterior

lobe of the pituitary gland (vehicle = 100%; Δ^9-tetrahydrocannabinol = 103%).

4. Discussion

The results of the present study demonstrate that 5 days daily administration with Δ^9-tetrahydrocannabinol increases POMC gene expression in the arcuate nucleus of the hypothalamus but was without effect in the anterior lobe of the pituitary gland. Activation of opioid gene expression was also found in a previous report (Mailleux and Vanderhaeghen, 1994) showing an increase in proenkephalin gene expression in the caudate-putamen after 3-week treatment with Δ^9-tetrahydrocannabinol.

POMC gene processes a number of proteins including β-lipotropin, α-, β- and γ-melanocyte-stimulating hormones, adrenocorticotropin hormone (ACTH) and β-endorphin (Eipper and Mains, 1980). In the anterior pituitary, ACTH and β-lipotropin are the predominant POMC-related peptides, whereas in the arcuate nucleus of the hypothalamus β-endorphin and α-melanocyte-stimulating hormone are the major end products of POMC processing (Gramsch et al., 1980; Emerson and Eipper, 1986). In the present study, the increase in POMC gene expression in the arcuate nucleus of the hypothalamus but not in the anterior lobe of the pituitary, after repeated injections with Δ^9-tetrahydrocannabinol, suggests an increase in the synthesis of β-endorphin and α-melanocyte-stimulating hormone. Since the highest concentration of β-endorphin, a very potent endogenous opioid (Pert et al., 1981), is found in the hypothalamus (Emerson and Eipper, 1986), the increase of POMC gene expression by Δ^9-tetrahydrocannabinol in the arcuate nucleus strengthens the idea that a number of behavioral and endocrinological effects reported of Δ^9-tetrahydrocannabinol may be mediated through activation of endogenous opioid tone.

Whether the increase in POMC expression induced by Δ^9-tetrahydrocannabinol in the arcuate nucleus is receptor mediated remains to be elucidated when cannabinoid antagonists become commercially available.

The dose of Δ^9-tetrahydrocannabinol selected in this study has been reported to induce significant changes in locomotor behavior, endocrine secretion and is in the range of the content of a cigarette of cannabis (Mailleux et al., 1994). The results of this study suggest that a short-term (5 days) exposure to cannabis might induce long-term effects on the opioidergic neuronal system in the brain.

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