Effect of acute administration of Δ^1-tetrahydrocannabinol on β-endorphin levels in plasma and brain tissue of the rat

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Summary. Acute treatment with Δ^1-tetrahydrocannabinol (Δ^1-THC) elevated the concentration of β-endorphin-like immunoreactivity (β-ELIR) in plasma and in the hypothalamus, but not in the hippocampus of rats habituated to the injection procedure. These effects were not obtained with the psychotropically inert analog of Δ^1-THC, cannabidiol. In animals that had not been habituated to the injection procedure, placebo treatment induced a decrease in hippocampal β-ELIR.
Key words. Δ^1-Tetrahydrocannabinol (Δ^1-THC); cannabidiol; beta-endorphin; plasma; brain; hypothalamus; hippocampus; rat.

Δ^1-Tetrahydrocannabinol (Δ^1-THC), the major psychoactive component of marihuana, has been shown to produce a variety of behavioral effects in laboratory animals[3–5]. We have recently found that Δ^1-THC induces a bizarre stereotyped excitatory circling and rotating response in rats after systemic administration in dosages of 3–12 mg/kg, even when it has a depressant effect on almost all behavioral elements normally occurring in an open field[6]. Since this effect could not be induced by the psychotropically inert analog of Δ^1-THC, cannabidiol (CBD)[7], it was interpreted as being related to the psychoactive properties of Δ^1-THC. In addition, Δ^1-THC but not CBD induced profound and selective effects on social interactions of rats that had been isolated for seven days and tested in dyadic encounters with non-isolated, untreated test partners[8].

β-Endorphin (β-E) and related peptides (in this article referred to as 'endorphins') were originally identified as peptides with pronounced opiate-like properties[9,10]. An extensive literature now exists substantiating a wide variety of effects of endorphins on animal behavior, ranging from induction of specific behaviors and behavioral activation to depression and immobility[11,12]. The endorphins are synthesized in the anterior and intermediate lobes of the pituitary, from which they can be released into the circulation[13,14]. In addition, they are found in neurons originating in the hypothalamus, with extensive projections throughout the brain[15,16]. In the central nervous system the endorphins are thought to function as neurotransmitters or neuromodulators, probably playing a role in the regulation of mood, drives and adaptive processes[11,12,17].

The aim of the present study was to investigate whether endogenous endorphin systems may be involved in the psychotropic effects of acute administration of Δ^1-THC or its psychotropically inert analog CBD on the concentration of β-endorphin-like immunoreactivity (β-ELIR) in plasma, hypothalamus and hippocampus of rats.

Materials and methods. Animals. Male albino Wistar rats weighing 160–180 g (Cpb:WU, bred from own stock) were transferred from the breeding accommodation to the experiment room one day before commencing the study. They were kept four to a cage in alternating 14 h light, 10 h darkness (lights on at 07.00 h), with free access to food and water.

Drugs. Ampoules containing pure natural trans Δ^1-tetrahydrocannabinol (Δ^1-THC) or cannabidiol (CBD) in dehydrated alcohol solution were received from the Laboratory of Natural Products of the Hebrew University, Jerusalem (Professor R. Mechoulam). The alcohol was evaporated under a nitrogen stream and the drug residue was dissolved in propylene glycol and suspended 1:5 (v/v) in a saline solution containing 2.2% Tween 80.

Treatment. On the three consecutive days preceding the experiment, three groups of animals (n = 8) were injected with saline (0.5 ml daily; i.p.) to habituate the animals to the injection procedure. A fourth group did not receive saline injections, and was left undisturbed during this period. On the day of the experiment, the habituated groups were treated with Δ^1-THC (2 mg/rat; approximately 12 mg/kg), CBD (4 mg/rat; approximately 24 mg/kg) or solvent (saline containing 2.2% Tween 80 and 20% propylene glycol). The non-habituated group received a solvent injection. All injections were performed i.p. in a volume of 0.5 ml between 11.00 h and 13.00 h. The animals were decapitated 45 min after the treatment.

Tissue dissection. Immediately after decapitation of the animals, the brain was rapidly removed from the skull, and placed on an ice-cooled PVC plate. Then, hypothalamus and hippocampus were dissected according to Gispen et al.[18]. In short, a transverse section was made through the chiasma opticum transecting the commissura anterior. The hypothalamus, defined as the region ventral to the commissura anterior, bordered laterally by the hypothalamic fissures and caudally by the corpora mammillare, was dissected with sharp forceps. Subsequently, the remaining diencephalic and mesencephalic tissue was removed, and the hippocampal complex was rolled out of the cortex in a caudal direction. The hippocampus was obtained after removal of fimbria and dentate gyrus. Tissues were immediately frozen on dry ice. Plasma was prepared by centrifugation of truncal blood, in chilled heparinized tubes. Both plasma and tissue samples were stored at – 80 °C until further processing.

Extraction of tissue and plasma. Tissues were heated for 10 min in 1 N acetic acid (1:10 w/v) in a boiling water bath, cooled on ice and homogenized by ultrasound. The resulting precipitate was removed by centrifugation. The supernatant was lyophilized and the residue dissolved in 1.0 ml phosphate-buffered saline (pH 7.5). Subsequently, endorphins were extracted and concentrated according to the method described by Ratcliffe and Edwards[19] with slight modifications. In short, heat-activated glass beads (Vycor®) were added to tissue extracts (35 mg/ml) and rotated for 4 h at 4 °C. The glass beads were collected by centrifugation and washed consecutively with distilled water and 1 N HCl. Then, peptides were eluted from the beads by rotation (30 min, 4 °C) with 60% acetone containing 1% acetic acid. The acetone fraction was collected by centrifugation, dried under nitrogen and dissolved in radioimmunoassay buffer. Plasma samples were treated similarly with Vycor® to extract endorphins. The recovery of the extraction procedure was determined with iodinated human β-endorphin (β-E), and amounted to 70–80%.

β-Endorphin radioimmunoassay. Radioimmunoassays were performed using an antiserum (B4) raised in rabbits against synthetic human β-E, synthetic human β-E as standard, and iodinated β-E as tracer. The sensitivity of the assay was 2 pg per tube at 10% displacement. The antiserum specifically recognized the midsequence of the β-E molecule. The following cross-reactivity data were obtained (on a mass basis at 50% displacement): human β-LPH, 39%; camel β-E(1–31), 110%; camel β-E(1–27), 100%; β-E(1–17), 450%; β-E(1–16), 200%; met-enkephalin, < 0.2%; α-MSH, < 0.8%. All assays were performed in duplicate in at least two dilutions of the sample. Sample dilution curves paralleled the standard curve. Total β-ELIR recovereed was computed for each sample. No corrections were made for recovery. β-ELIR concentrations are presented as pg/ml (plasma) or ng/tissue (brain regions).

Statistics. The data were statistically analyzed using a one-way analysis of variance (ANOVA), followed by a Newman-Keuls test.

Chemicals. All chemicals used were of analytical grade. The peptides used were synthesized and kindly provided by Organon International bv, Oss, The Netherlands.

Results. In animals treated with Δ^1-THC, plasma β-ELIR was significantly increased 45 min after the injection as compared with their habituated, solvent treated controls (fig. 1A). Plasma β-ELIR in the CBD treated animals did not differ from control values. Also, plasma β-ELIR levels in animals that received a solvent injection without being habituated to the injection procedure were not significantly different from those found in habituated solvent treated controls (fig., A).

The β-ELIR concentration in the hypothalamus of Δ^1-THC treated animals was increased 45 min after the administration of the drug, as compared to habituated controls (fig., B). Treatment with CBD did not significantly affect the β-ELIR concentration in the hypothalamus. No difference was found in hypothalamic β-ELIR between non-habituated solvent-treated animals and habituated controls (fig., B).

In animals treated with Δ^1-THC, the β-ELIR concentration in the hippocampus was slightly, but not significantly lower 45 min after the injection than that in habituated controls (fig., C). Administration of CBD had no effect on hippocampal β-ELIR levels. In solvent-treated animals that had not been habituated to the injection procedure, the β-ELIR concentration in the hippocampus was significantly lower than in the habituated controls (fig., C).

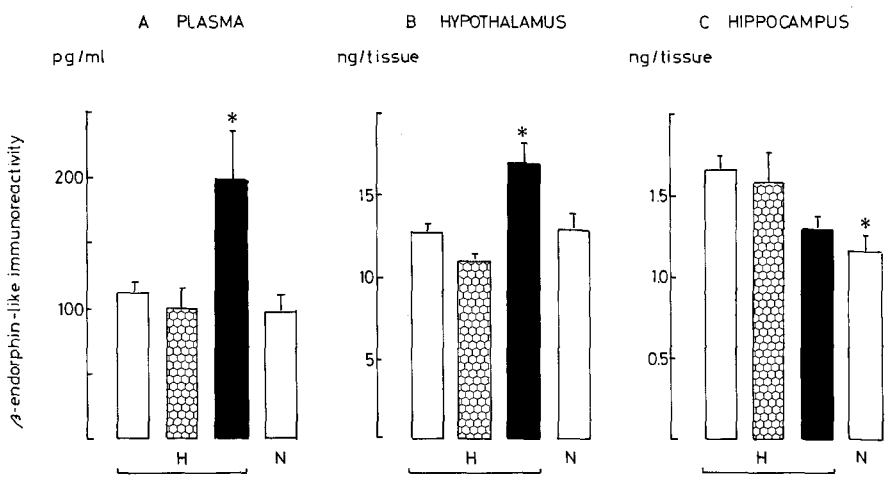
Discussion. In order to determine the effects of cannabinoids on β-ELIR levels in plasma and brain tissue, we used an antiserum specifically recognizing the midportion of the β-E molecule. All peptides containing the sequence β-E(9–16) tested so far, including β-LPH, displayed considerable cross-reactivity with this antiserum (see 'Materials and methods'). Thus, our radioimmunoassay system most likely detects all forms of αE, βE and γE present in plasma and tissue samples, as well as the precursor molecules of these peptides. The data therefore represent total β-endorphin-like immunoreactivity (β-ELIR).

In the present study, acute peripheral administration of Δ^1-THC elevated the concentration of β-ELIR in plasma and in the hypothalamus, but did not significantly affect hippocampal β-ELIR. These effects may be related to the psychoactive properties of Δ^1-THC, since its psychotropically inactive analog CBD[7] did not change β-ELIR levels.

β-Endorphin and related peptides are synthesized in and released from the corticotrophs of the anterior lobe and in the melanotrophs of the intermediate lobe of the pituitary[13,14]. From the present experiments, no conclusions can be drawn as to whether the increase in plasma β-ELIR found after treatment with Δ^1-THC reflects enhanced secretion from the anterior lobe, the intermediate lobe, or both. Abundant evidence is available, however, that Δ^1-THC stimulates ACTH release from the pituitary[20–22]. As ACTH, β-LPH and endorphins are derived from the same precursor molecule pro-opiomelancortin (POMC)[23,24], are colocalized in secretory granules[25], and are simultaneously released by corticotrophs[13,14,26], it is likely that the observed stimulatory effect of Δ^1-THC on plasma β-ELIR involves enhanced secretory activity of anterior pituitary cells.

Apart from a direct action of Δ^1-THC at the level of the pituitary, an interaction of the substance with central nervous structures that are involved in regulation of pituitary function may underly its effect on plasma β-ELIR. Indeed, Δ^1-THC probably acts on hypophysiotropic structures in the hypothalamus, thereby altering the release of several hormones from the anterior pituitary[27,28]. Moreover, it has been shown that Δ^1-THC selectively accumulates in the hippocampus in high concentrations[29], and that it inhibits the uptake of corticosteroids in the hippocampus in vivo[30]. Thus, a disruption of the negative feedback on the releasing activity of anterior pituitary corticotrophs that is mediated by hippocampal corticosteroid receptor mechanisms[31] may provide an alternative explanation for the effect of Δ^1-THC on pituitary β-ELIR release.

In the brain, endorphins are found in a neuronal system originating in the hypothalamus (nucleus arcuatus), with projections to structures within the hypothalamus and a number of extrahypothalamic regions of the brain, including the hippocampus[15,16]. POMC is synthesized in the cell bodies, and the enzymatic processing of this precursor to β-E and related peptides is thought to occur mainly inside the secretory granules during axonal transport[13]. Δ^1-THC increased the concentration of β-ELIR in the hypothalamus. In view of the relatively short latency of this effect (45 min), it is unlikely that it is brought about through a stimulation of POMC biosynthesis at the level of the cell bodies in the nucleus arcuatus. A more feasible explanation is that Δ^1-THC inhibited the release of endorphins from nerve endings in the hypothalamus, and in that way increased the local β-ELIR concentration. In contrast, hippocampal β-ELIR concentrations showed a slight but not statistically significant decrease. In the hippocampus, the localization of endorphins is exclusively synaptic[15,16]. The results therefore suggest that Δ^1-THC does not affect – or, if anything, enhances – the release from POMC neurons projecting in the hippocampus, thereby depleting synaptic stores in this area.



Concentration of β-endorphin-like immunoreactivity (β-ELIR) in plasma (A), hypothalamus (B) and hippocampus (C) 45 min after various treatments. Rats that had been habituated to the experimental procedure (daily i.p. saline injection on the three days preceding the experiment; bars marked H) and naive animals (bars marked N) were treated with solvent (open bars), cannabidiol (24 mg/kg; dotted bars) or Δ^1-tetrahydrocannabinol (12 mg/kg; closed bars). β-ELIR concentrations (mean±SEM, n = 7–8) were determined by radioimmunoassay on extracted samples. No corrections were made for recovery. Results of ANOVA testing: A: $F(3,27) = 5{,}19$ $p < 0.05$; B: $F(3,26) = 7{,}31$, $p < 0.05$; C: $F(3,28) = 4{,}56$, $p < 0.05$.
*Significantly different from habituated controls ($p < 0.05$; Student Newman-Keuls test).

Thus, from the present data it appears that Δ^1-THC differentially influences the release of β-ELIR in hypothalamus and hippocampus. In general, effects on release can be brought about either at the level of the terminals, or of the cell bodies of neurons. As the POMC-terminals in both brain regions arise from the same group of neurons located in the nucleus arcuatus, the differential effects of Δ^1-THC on β-ELIR in hypothalamus and hippocampus are best explained by actions of the drug at the level of the terminals. The difference in the effects on these brain regions may well be related to differences in neuronal connectivity in these areas. As yet, it remains unclear whether the effects of Δ^1-THC on brain β-ELIR are related to the effect on the release of endorphins from the pituitary.

Interestingly, hippocampal β-ELIR in solvent-treated animals that were not habituated to the injection procedure appeared to be significantly lower than that in habituated controls. The hippocampus plays an important role in the interpretation of novel environmental stimuli and adaptive behavior[32], and the observed difference in β-ELIR may therefore be related to the difference in novelty value of the experimental procedure experienced between the two groups of animals. Previous observations, that hippocampal β-ELIR levels are extremely dependent on the history of the animals with respect to environmental stimuli, are in line with this notion (Wiegant et al., unpublished).

Many similarities exist between the pharmacological effects of cannabinoids and of opioids. Both classes of substances can produce hypothermia[33,34], analgesia[35,36], stereotyped behavior, behavioral activation and depression[3-6,8,37-39] and interfere with the secretion of prolactin, gonadotropins and POMC-derived hormones from the pituitary[20-22,27,28]. Some of the effects of Δ^1-THC can be blocked by opiate receptor antagonists[39,40], suggesting that endogenous opioid systems are involved in actions of this substance. Indeed, endogenous endorphin systems have been implicated in the regulation of temperature, pain perception, pituitary function, mood, drives and adaptive behavior[11,12,17]. Our data that Δ^1-THC and not its psychotropically inert analog CBD acutely influences the activity of the endorphin systems in brain and pituitary, provide further support for a role of endorphins as mediators of psychotropic actions of Δ^1-THC. Whether the effects observed result from a direct interaction of Δ^1-THC with POMC synthesizing cells or occur through (an) indirect mechanisms involving other neurotransmitters, remains to be established.

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