MODULATORY EFFECT OF CANNABINOID LIGANDS ON THE ANXIETY-LIKE BEHAVIOR OF BULBECTOMIZED RATS

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ABSTRACT:

Purpose: The endocannabinoid system is considered a key regulatory system in anxiety behavior. The aim of the present study was to examine the effects of intracerebroventricularly (i.c.v.) injected cannabinoid ligands on the anxiety-like behavior of rats with a model of depression.

Material/Methods: The olfactory bulbectomized rat (OBX) is a well-established experimental model of depression. The OBX model exhibits neurochemical changes that are very similar to those seen in patients with depression. CB1 receptor agonist HU-210 and CB1 receptor antagonist SR 141716A were injected i.c.v. in OBX rats, and the anxiety-related behavior of the rats was measured in an elevated plus-maze (EPM) test.

Results: OBX rats showed an increased anxiety-like behavior at the EPM test. HU-210 produced an anxiolytic-like effect and alleviated the OBX-induced anxiety, while SR 141716A failed to produce effects on the behavior of OBX rats.

Conclusions: The results suggest that CB1 receptors may be involved in the modulation of anxiety-related behavior in OBX rats.

Key words: Endocannabinoid system, Olfactory bulbectomy, Anxiety, Depression, HU-210, SR 141716A.

INTRODUCTION

Extracts of *Cannabis sativa* (marijuana) have been used for their calming and sedative effects for centuries [1]. Marijuana intake causes relaxation and gives one a sense of well-being. Heavy use of marijuana has also been linked to addiction, anxiety, and panic disorders [2].

The endocannabinoid system is a lipid signaling system in the brain that consists of endocannabinoids, cannabinoid receptors and the enzymes involved in the endocannabinoid biosynthesis or inactivation. The endocannabinoids are derivatives of arachidonic acid, and the two major endogenous ligands are anandamide (N

arachidonylethanolamine) and 2-arachidonoylglycerol. There are two types of cannabinoid receptors (CB1 and CB2), which belong to the family of G-protein-coupled receptors. Endocannabinoids are synthesized postsynaptically from lipid membrane precursor molecules and act presynaptically on CB1 receptors by modulating the release of neurotransmitters in the central nervous system. The endocannabinoid signaling has been implicated in the modulation of feeding behavior, pain, cognitive processes [3] as well as in the regulation of the behavioral responses to stress [4].

The endocannabinoid system plays an important role in the pathogenesis of depressive disorders, which are known to be accompanied by impaired cognitive functions and anxiety. Research has suggested that modulation of neuronal endogenous cannabinoid signaling systems could represent a novel approach to the treatment of anxiety-related disorders [5].

Bilateral olfactory bulbectomy (OBX) is an experimental model of depression, which produces in rodents a syndrome with behavioral, neurochemical, and structural abnormalities similar to those observed in human depression [6].

The aim of our study was to examine the effect of centrally administered CB1 receptor ligands on the anxiety of rats with OBX model of depression.

MATERIALS AND METHODS

Animals. The experiments were carried out on 32 male Wistar rats (200-220 g).

Experimental model of depression – bilateral olfactory bulbectomy (OBX). The surgical procedure involved drilling two burr holes were performed in stereotaxic apparatus (Narishige S.I.L.), according to coordinates to the stereotaxic atlas of Pellegrino and Cushman [7]. The bulbs were aspirated with a stainless needle attached to a water pump.

Stereotaxic implantation and drug injection. After anesthesia, the rats were placed in the stereotaxic appa-

ratus (Narishige S.I.L.) and guide cannulae were implanted into ventriculus ventrolateralis dextra, according to coordinates to the stereotaxic atlas of Pellegrino and Cushman [7]. HU-210 (Tocris) and SR 141716A (Sanofi) were dissolved ex tempore in a 1:19 solution of dimethyl sulfoxide/0.9 % saline and 1 μ l of drug solution (pH 7.4) was infused into ventriculus ventrolateralis dextra 5 min before the behavior test.

Behavioral methods: Elevated plus maze (EPM) test. The plus maze is consisted of two open arms (50 x 10 cm), facing each other, and two closed arms (50 x 50 x 40 cm), and was elevated to a height of 50 cm. EPM test was performed according to the method described by Pellow et al. [8]. The number of entries in the open arms and closed arms of the apparatus and the time spent in the open arms was measured during the five-minute test.

Statistical analysis. One-way ANOVA was used to process the data obtained for learning and memory. Data were further analyzed by post hoc t-test where appropriate.

RESULTS

ANOVA analysis showed a significance for the depression factor in OBX rats, concerning the number of open arms entries ($F_{1,23} = 14.57$; $P \leq 0.001$), time spent in the open arms ($F_{1,23} = 100.92$; $P \leq 0.001$) and the ratio open/total number of entries ($F_{1,23} = 22.37$; $P \leq 0.001$). The OBX rats demonstrated a decreased number of entries in the open arms ($t = 3.82$; $P \leq 0.001$) and time spent there ($t = 10.05$; $P \leq 0.001$), and decreased ratio open/total number of entries ($t = 4.73$; $P \leq 0.001$) (Fig. 1, 2, 3).

In the drug-treated OBX group ANOVA revealed a significance for the drug factor for the number of open arms entries ($F_{2,35} = 8.97$; $P \leq 0.001$), time spent there ($F_{2,35} = 39.14$; $P \leq 0.001$), and the ratio open/total number of entries ($F_{2,35} = 37.90$; $P \leq 0.001$). HU-210 significantly increased the number of open arms entries ($t = 3.08$; $P \leq 0.01$), time spent in the open arms ($t = 6.18$; $P \leq 0.001$), and increased the ratio open/total number of entries ($t = 6.56$; $P \leq 0.001$) as compared to the OBX-saline treated rats. (Fig. 1, 2, 3). SR 141716A did not affect significantly the behavior of OBX rats in the EPM as compared to the controls. (Fig. 1, 2, 3).

Fig. 1. Effects of HU-210 (5 μ g) and SR 141716A (3 μ g), microinjected i.c.v., on the number of open arm entries in EPM test. Means ($\pm$ S.E.M.) are presented. $^{\circ\circ\circ}$ $P \leq 0.001$ – comparison vs sham-operated controls. *** $P \leq 0.01$ – comparisons vs saline-treated OBX controls.

Fig. 2. Effects of HU-210 (5 µg) and SR 141716A (3 µg), microinjected i.c.v., on time spent in the open arms in EPM test. Means (± S.E.M.) are presented. °°° P ≤ 0.001 – comparison vs sham-operated controls. *** P ≤ 0.001 – comparisons vs saline-treated OBX controls.

Fig. 3. Effects of HU-210 (5 µg) and SR 141716A (3 µg), microinjected i.c.v., on the ratio open/total number of entries in EPM test. ° P ≤ 0.05, °°° P ≤ 0.001 – comparison vs sham-operated controls. *** P ≤ 0.001 – comparisons vs saline-treated OBX controls.

DISCUSSION

The results of the present study demonstrate that the activation of the brain CB1 receptors modulates the anxiety-like behavior of the OBX rats so that the tested parameters approached the normal values of the sham-operated controls. However, the CB1 antagonist SR 141716A showed no significant behavioral effects.

So far it is difficult to evaluate the effects of the cannabinoid treatment in terms of its application in clinical practice. The reports vary, and it seems that the effects depend on the dose and route of administration of the cannabinoid ligands. While the low doses cannabinoid agonists usually have an anxiolytic effect, the high doses may be anxiogenic [9]. Lisboa et al. found anxiolytic effect after intraperitoneal (i.p.) administration of the CB1 agonist WIN55, 212-2 in a model of repeated social defeat in mice [10], and Alteba et al. in a model of early life stress [11]. The i.p. injection of WIN55, 212-2 resulted in an antidepressant effect in mice with social isolation stress [12], however, the administration of higher doses HU-210 in an acute stress model exhibited an anxiogenic effect [13]. In addition, the effects of the CB1 receptor activation may be different when applied to different brain structures. For example, cannabidiol injection into the ventral medial prefrontal cortex produces an antidepressant effect [14], while WIN55, 212-2 injections into the lateral septum has an anxiogenic effect [15].

One of the most popular theories about the pathogenesis of the depressive disorder is the monoaminergic hypothesis, which suggests the presence of dysfunction in serotonergic and noradrenergic neurotransmission. The improvement of monoaminergic neurotransmission is

an effect typical of all antidepressants, regardless of their mechanism of action [16]. The interactions between the endocannabinoid and the serotonergic system in the brain have been demonstrated in experimental studies. Recently, a modulatory effect of CB1 receptor agonists on the 5-HT neuronal activity in the ventromedial prefrontal cortex has been demonstrated [17]. Low doses of WIN55, 212-2 potentiated neuronal activity in nucleus raphe dorsalis in the brainstem and the established anti-depressive effect was associated with activation of the endocannabinoid system in the prefrontal cortex [17]. Blockade of CB1 receptors with SR 141716A, microinjection of cannabidiol, as well as the chronic administration of tetrahydrocannabinol, increased the serotonin levels in the prefrontal cortex [14, 18, 19], while the chronic peripheral administration of HU-210 potentiated behavioral effects in response to single-acting 5-HT1A and 5-HT2A receptor agonists [20].

Based on the results obtained, we can assume that the CB1 receptor signaling is most likely involved in the mechanisms of the state of increased anxiety induced by the olfactory bulbectomy.

CONCLUSIONS

1. In the rats with OBX model of depression, HU-210 produced an anxiolytic-like effect, alleviating OBX-induced anxiety. However, SR 141716A failed to produce any effects on the behaviour of OBX rats in the elevated plus maze test.

2. The results point to a modulatory effect of the cannabinoid ligands on the anxiety-like behavior of OBX rats in the elevated plus maze test.

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