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EFFECTS OF ACUTELY APPLIED CANNABINOID CB1 LIGANDS ON NOCICEPTION IN RATS WITH A MODEL OF DEPRESSION

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Abstract

The effects of CB1 receptor agonist HU-210 and cannabinoid CB1 receptor antagonist SR 141716A on nociception of male Wistar rats with a model of depression (bilateral olfactory bulbectomy, OBX) were studied. HU-210 (5 µg) and SR 141716A (3 µg) were acutely microinjected i.c.v. on the developed depression background. Nociception was examined as applied mechanical pressure on the left hind paw of the rat (analgesy-meter test, Randall & Sellitto). In the OBX rats, it was found that the pain threshold was increased. HU-210 significantly increased the pain threshold in OBX rats, i.e. decreased the pain sensitivity as compared to saline-treated OBX controls and to sham-operated controls, suggesting an implication of CB1 receptors in nociception of OBX rats. SR 141716A did not affect the nociception in OBX rats. These results suggest that stimulation of CB1 receptors in rats with a model of depression exerted antinociceptive effect.

Key words: CB1 cannabinoid receptors, nociception, olfactory bulbectomy, depression, rat

Introduction. The bilateral olfactory bulbectomy (OBX) syndrome in rats has been proposed as an animal model of chronic depression. Bilateral olfactory bulbectomy is associated with changes in behaviour, and in the endocrine, immune, and neurotransmitter systems, which simulate many of those seen in patients with major depression.

In the bulbectomized rats, some of the neurochemical and behavioural changes are similar to those observed in depressed patients. Recently, the role of

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cannabinoid receptors has attracted much attention in relation to diverse neuropsychiatric disorders such as major depression.

Two cannnabinoid receptors have been identified and molecularly characterized so far, both G protein coupled – cannabinoid receptor type 1 (CB1 receptor) and type2 (CB2 receptor) [1, 2]. It is thought that the effects of cannabinoids on the central nervous system (CNS) are mediated by the CB1 receptor. CB1 receptors are widely expressed in the brain, including the olfactory bulb, cortical regions (neocortex, pyriform cortex, hippocampus and amygdale, parts of basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex and brainstem nuclei, including PAG) [3]. In the brain, CB1 receptors are targeted by endogenous cannabinoids (i.e. endocannabinoids), such as anandamide (AEA), 2-arachidonylglycerol and arachidonylethanolamide [4]. The synthetic derivative HU210 shows the highest potency among the CB1 receptor agonists, whereas SR 141716A shows the highest potency as CB1 receptor antagonist.

The complex physiological function of the cannabinoid system includes locomotor coordination, muscle relaxation, cognition, bronchial dilatation, pain alleviation, anti-allergic and anti-inflammatory effects, appetite stimulation, neural protection, etc. [5]. We have found that stimulation of CB1 receptors suppresses exploratory and locomotor activity, while CB1 receptors inhibition stimulates them [6].

The aim of the present study was to investigate the effects of CB1 receptors agonist HU-210 and antagonist SR 141716A, acutely applied i.c.v., on nociception in rats with a model of depression (OBX).

Materials and methods. ***Animals.*** The experiments were carried out on 84 male Wistar rats (200–240 g at the time of surgery). The experiments were performed according to the "Rules for care and experiments on laboratory animals of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Experimental model of depression – bilateral olfactory bulbectomy (OBX). Bilateral olfactory bulbectomy was performed according to the method described by Kelly et al. [7]. Animals were anaesthetized with Calypsol (50 mg/kg i.p.). The top of the skull was shaved and swabbed with an antiseptic, after which the animals were placed in a stereotaxic apparatus (Stoelting, USA). The surgical procedure involved drilling two burr holes 2 mm in diameter, at the points of 8 mm anterior to bregma and 2 mm from the midline on both its sides (coordinates of bulbi olfactorii were detected according to the stereotaxic atlas of Pellegrino and Cushman [8]). The bulbs were aspirated with a stainless needle attached to a water pump. The cavity was packed with Gelaspon used as a haemostatic.

After the surgery, the rats were housed in groups of two and were handled and weighed daily during a 15-day period. The sham operation was performed in the same way as in the case of olfactory bulbectomy, without the removal of the olfactory bulbs.

Stereotaxic implantation and drug injection into ventriculus ventrolateralis dextra. After anaesthesia (Calypsol 50 mg/kg i.p.), the rats were placed in a stereotaxic apparatus (Stolting, USA) and guide cannulae were implanted into ventriculus ventrolateralis dextra (P = 0.9 mm; R = 1.6 mm; h = −3.0 mm) according to the coordinates of the stereotaxic atlas of Pellegrino and Cushman [8]. HU-210 [3-(1,1-dimethylheptyl)-(-)-11-hydroxy-delta 8-tetrahydrocannabinol] (Tocris) and SR 141716A [N-piperidino-5-(4-chlorophenyl)1-(2,4-dichloro-phenyl)-4-methyl pyrazole-3-carboxamide hydrochloride] (Sanofi) were dissolved ex tempore in a 1:19 solution of dimethyl sulfoxide/0.9% saline and 1 µl of drug solution (pH 7.4) was infused i.c.v. 5 min before the behavioural test. Following the termination of the experiments and immediately prior to sacrificing, the rats were injected with 1 ml 2% fast green dye through the injection cannula for verification.

Paw-pressure test. Nociception was examined applying mechanical pressure on the left hind paw of the rat and calculated in arbitrary units, according to Randall and Selitto method [9].

The animals were divided in six groups: I – three groups, which were injected i.c.v. with either HU-210 (5 µg), or SR141716A (3 µg), or saline. II – four groups OBX rats: OBX + saline, OBX + HU 210, OBX + SR141716, and sham operated rats.

Statistical analysis. Data were analysed by one-way ANOVA. Separate one-way was used to process the data obtained for pain threshold (AU) after injected HU-210 or SR141716A, and HU-210 or SR141716A infused on OBX rats. ANOVA data were further analyzed by post-hoc Student-Newman-Keuls test, where appropriate.

Results. ***Effects of HU-210 and SR 141716A on nociception.*** One-way ANOVA of the effects of HU-210 and SR 141716A on pain threshold after i.c.v. injections of HU-210 or SR 141716A demonstrated a significant effect for factor drug ($F_{2,35}$ = 54.465; $P \leq 0.001$). Post-hoc comparisons of HU-210 (5 µg) and SR141716A (3 µg) i.c.v. acutely injected rats and controls indicated that HU-210 significantly increased the pain threshold (t = 8.578; $P \leq 0.001$), while acute i.c.v. injections of SR141716A did not significantly changed the pain threshold (t = 0.685, P = NS) (Fig. 1).

Effects of HU-210 and SR 141716A infused on the background of developed depression. The bilateral removal of the rat bulbi olfactorii resulted in an increase of the pain threshold in the paw-pressure test. One-way ANOVA analysis of the changes in the nociceptive responses demonstrated a significant effect for the pain threshold in OBX rats ($F_{1,14}$ = 52.744; $P \leq 0.001$). Post-hoc comparisons showed that in OBX rats the pain threshold was increased (t = 6.70; $P \leq 0.001$), as compared to the sham operated controls (Fig. 1).

One-way ANOVA showed a significant effect for drugs ($F_{3,47}$ = 11.646; $P \leq 0.001$) after acute i.c.v. microinjections of HU-210 and SR 141716A. Post-hoc

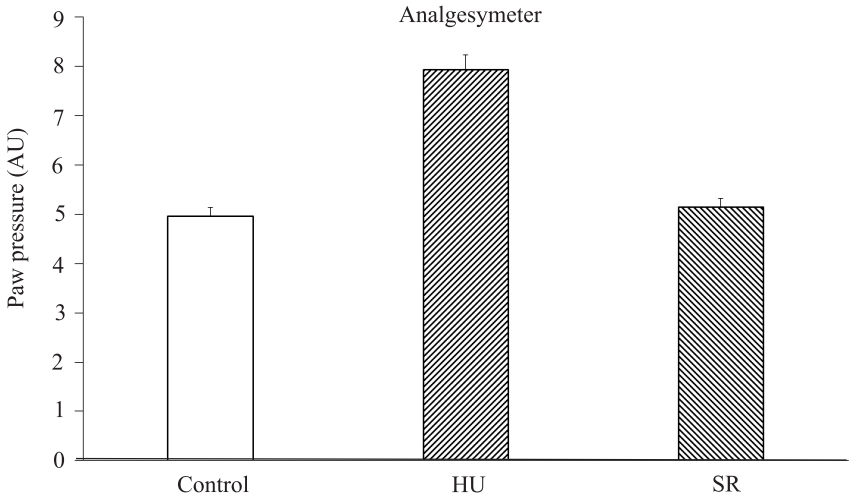

Fig. 1. Effects of HU-210 (5 µg) and SR 141716A (3 µg), microinjected i.c.v., on pain threshold (Arbitrary Units – AU). $n = 12$. $^{***}P \leq 0.001$ – comparisons vs saline-treated controls. Means (± S.E.M.) are presented

NSK comparisons demonstrated that infused at a dose of 5 µg HU-210 produced a significant increase of the pain threshold in OBX rats, as compared to the OBX saline-treated rats ($t = 3.99$, $P \leq 0.001$) (Fig. 2), while i.c.v. injections of SR 141716A (3 µg) did not show significant changes in the pain threshold, as compared to the OBX saline-treated rats ($t = 1.27$; $P =$ NS). However, compared to the sham-operated rats, HU-210 ($t = 14.023$; $P \leq 0.001$) and SR 141716A ($t = 4.994$; $P \leq 0.001$) significantly increased the pain threshold (Fig. 2).

Discussion. The present study showed that acute i.c.v. infusion of CB1 agonist HU-210 increased the pain threshold, while CB1 antagonist SR 141716A did not affect it. Analgesic effects of cannabinoids have been demonstrated in most animal models of pain. The antinociceptive effects involve actions at different levels, including peripheral sensory neurons, spinal cord, and central pathways [10,11]. CB1 receptors are present in brain areas involved in nociception, such as thalamus and amygdala [12, 13]. CB1 receptors are also expressed in the cells of the midbrain periaqueductal grey matter (PAG), and in the substantia gelatinosa of the spinal cord (receiving nociceptive input from primary afferent neurons), which are key sites for modulating nociceptive information [14, 15]. There are data that both systemic and i.c.v. administration of cannabinoid receptor agonists produce analgesia [16, 17] involving central antinociceptive mechanisms. CB1 receptor agonists produce antinociception in spinal and supraspinal sites of action, as investigated in intact rats, while the CB1-specific antagonist SR141716A facilitates nociceptive responses [18, 19].

Depression and painful somatic symptoms commonly occur together and complex interactions between pain perception and depressive symptoms have been described. Bilateral olfactory bulbectomy produces a syndrome of behavioural,

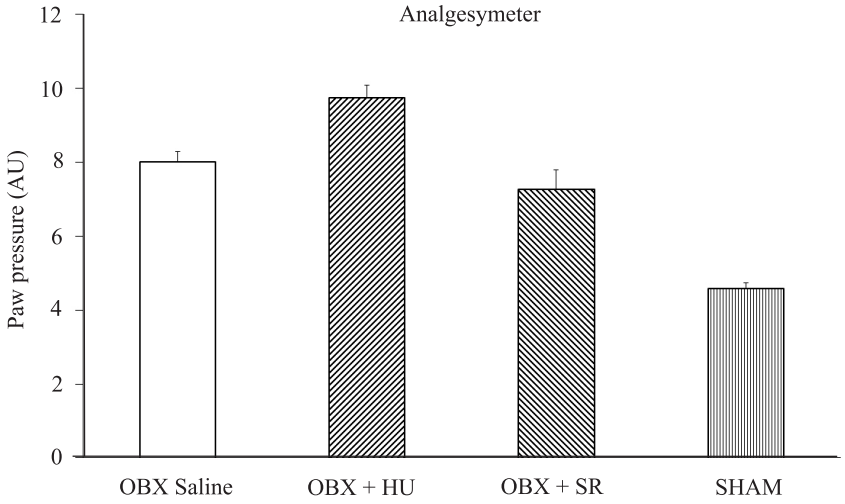

Fig. 2. Effects of HU-210 (5 µg) and SR141716A (3 µg), microinjected i.c.v., on nociception in OBX rats. Asterisks depict comparisons of pain threshold (AU) in HU-210-treated OBX rats or SR141716A-treated OBX rats vs. OBX saline-treated rats. $^{***}P \leq 0.001$. Circles depict comparisons after OBX rats microinjected with HU-210 or SR141716A vs. sham operated saline-treated rats. $n = 12$. $^{\circ\circ\circ}P \leq 0.001$. Means (± S.E.M.) are presented

neurochemical and pathophysiological alterations that resemble human depressive disorders. In our previous studies, using paw-pressure method, we have found that the pain threshold of OBX rats has increased compared to the sham operated controls (Belcheva) [20].

To our knowledge, the present study is the first to investigate the effects of CB1 receptors on nociception of rats with a model of depression, using paw-pressure method. The important finding was that HU-210 microinjected i.c.v. on the background of developed depression-like behaviour increased the pain threshold in OBX rats compared with saline-treated OBX controls and with sham-operated controls. SR 141716A increased the pain threshold in OBX rats only compared to the sham-operated controls.

The interpretation of this finding is difficult but involvement of the CB1 receptors in the nociceptive response of OBX rats may be suggested. Moreover, stimulation of CB1 receptors decreased the pain sensitivity in depressed rats, while its inhibition did not affect nociception.

In conclusion, these results suggest that stimulation of CB1 receptors in rats with a model of depression exerts antinociceptive effect.

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