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Hemispheric asymmetry in learning and memory after unilateral infusion of cannabinoid ligands into the CA1 hippocampal area of bulbectomized rats

R. E. Tashev[1], Hr. H. Nocheva[1], M. S. Velikova[2]*

[1]*Department of Physiology and Pathophysiology, Medical University of Sofia, 2, Zdrave Str., 1431 Sofia, Bulgaria*
[2]*Department of Physiology and Pathophysiology, Medical University of Varna, 55, M. Drinov Str.,* 9002 *Varna, Bulgaria*

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The effects of the ligands of cannabinoid receptor HU 210 (CB-receptor agonist) and SR 141716A (CB1 receptor antagonist) infused unilaterally (left or right) into the CA1 hippocampal area on learning and memory (two-way active avoidance task) in rats with a depression model (olfactory bulbectomy, OBX) were examined. We found that HU 210 (5 μg/ 0.1 μl) microinjected unilaterally into the CA1 area of rats impaired learning and memory processes, the effect being more pronounced on the right side. SR 141716A (3 μg/0.1 μl) infused into the left or right CA1 area had no significant effect on (sham-operated) rat performance in the active avoidance test. The most important findings are that the administration of HU 210 in the right CA1 area reversed the OBX-induced learning and memory deficits, while microinjection into the left CA1 area ameliorated the acquisition and retention deficits in OBX rats. SR 141716A further impaired the rat's performance and its suppressive effect was present only on the right side. The results demonstrate that CB receptors are involved in learning and memory in OBX rats. Our study provides for the first time, data about differential and asymmetrical effects of cannabinoid ligands on learning and memory in OBX rats, suggesting a lateralization of CB receptors in the brain hemispheres. The asymmetrical distribution of CB receptors in the left and right CA1 hippocampal area could contribute to the asymmetry in the cognitive effects of the CB receptor ligands.

Keywords: CB ligands, hemispheric asymmetry, learning and memory, depression

INTRODUCTION

Hemispheric asymmetry, characterized by differences between left and right hemispheres, exists on structural, functional, and molecular levels [1, 2]. Evidence supports the presence of receptor or structural asymmetry in pathways mediating behavioral responses, contributing to lateralized behavioral patterns. Neuropharmacological studies have explored asymmetry in behavioral responses (such as exploratory behavior, motor activity, anxiety, pain perception, and learning and memory) across various brain structures, and have established asymmetry in neurotransmitter systems such as dopamine, gamma-aminobutyric acid, serotonin neurotransmitter system, as well as asymmetry involving neuropeptides - cholecystokinin, somatostatin, vasoactive intestinal peptide, and angiotensin [3-5].

Studies have found significant alterations in brain regions in depressive disorder patients, such as the hippocampus, amygdala, and prefrontal and frontal cortex; similar changes have been observed in OBX rats [6-8]. The hippocampus, a limbic system structure, plays a crucial role in depression. Patients with major depression often exhibit reduced hippocampal volume; structural and functional changes in the hippocampus have been linked to cognitive impairment. In addition to its role in memory formation, recent studies have associated the hippocampus with emotional and cognitive functions.

Bilateral olfactory bulbectomy (OBX) is a valid animal model for depression and it is also used for modeling Alzheimer's disease. Following OBX, rats display behavioral changes including hyperlocomotion, memory disturbances, reduced sexual activity, aggressive behavior, and hyperemotionality [8]. The correlation between structural, functional, and biochemical alterations in the brains of both depressed patients and OBX rats supports the use of OBX as a model to study the mechanisms underlying the pathophysiology of depression.

Accumulating evidence has underscored the significance of the endocannabinoid system in modulating behavioral, neurochemical, neuroendocrine, and neuroimmune responses to various stimuli [9]. Cannabinoids have been shown to alleviate hippocampal neuronal loss following cerebral ischemia and acute brain injury, providing neuroprotection in neurodegenerative conditions

* To whom all correspondence should be sent:
E-mail*: Margarita.Stefanova@mu-varna.bg*

including Alzheimer's, Parkinson's disease, multiple sclerosis, Huntington's chorea, Tourette's syndrome, epilepsy, stroke, and various dementias [10]. Several mechanisms have been proposed to elucidate the neuroprotective effects of cannabinoids, including neuromodulation and calcium homeostasis modulation, protein kinase activation, and antioxidant activity, among others [10, 11]. Findings suggest the involvement of endogenous cannabinoids in regulating cognitive processes. Moreover, the endocannabinoid system has been implicated in the pathogenesis of depressive disorders which often manifest with cognitive impairment, including memory deficits [12, 13]. The two major cannabinoid receptors (CB1 and CB2) belong to the family of G-protein coupled receptors [10]. CB1 receptors are densely distributed in brain areas related to motor control, cognition, emotional responses, and motivated behavior [10, 12].

The relationship between hemispheric brain asymmetry and depression has been actively investigated, and abnormal asymmetry has also been reported to be associated with poor cognitive function. Previously, we demonstrated impaired learning and memory in OBX rats after intracerebroventricular (i.c.v) application of CB receptor ligands (HU 210 and SR 141716A) using both active and passive avoidance tests [14, 15]. HU 210 improved the memory deficits of OBX rats, while SR 141716A impaired their performance. Based on the data mentioned above, this study aimed to investigate the hemispheric asymmetry in learning and memory in response to unilateral infusion of the CB receptor agonist (HU 210) and the CB1 receptor antagonist (SR 141716A) into the CA1 hippocampal area in rats with a depression model, using a two-way active avoidance test.

EXPERIMENTAL

Animals

The experiments were carried out on 42 male Wistar rats weighing 200–220 g at the time of surgery. Behavioral experiments were performed between 10:00 am and 1:00 pm. The experiments were conducted following the guidelines of the local Ethics Committee, which fully comply with EC Directive 2010/63/EU for animal experiments. All efforts were made to minimize animal suffering and reduce the number of animals used in the study.

Surgical procedures. Experimental model of depression (OBX). Stereotaxic implantation and drug microinjection into the CA1 hippocampal area

Bilateral olfactory bulbectomy (OBX) was carried out according to the method described by Kelly *et al.* [16]. Seven days after bilateral olfactory bulbectomy cannulae were surgically implanted bilaterally into the CA1 area as described previously [5]. After cannulae implantation, the rats were allowed 7 days to recover. HU-210 (Tocris) and SR 141716A (Sanofi) were dissolved *ex tempore* in saline and 0.1 µl of HU-210 (5 µg) or 0.1 µl of SR 141716A (3 µg) (pH 7.4) were microinjected over 1 min into the left or right CA1 hippocampal area. The injection cannula was left in place for an additional 30 sec. Five min after the infusion the rats were tested in the shuttle box. Sham-operated rats were subjected to a procedure identical to that described for the OBX rats.

Learning and memory test

- *Two-way active avoidance (shuttle-box).* The behavioral tests were performed 14 days after OBX-surgery, i.e. when a depressive-like state was developed. The animals were tested in a two-way active avoidance test (shuttle box) described previously [5]. HU-210, SR 141716A, or saline were infused 5 min before the tests.
- *Verification*. Before the scarification, the rats were microinjected into the CA1 hippocampal area with 1µl 2% Fastgreen dye. Bulbectomy and the injection sites were verified *post-mortem*. Animals with misplaced or asymmetrical cannulas were excluded from the statistical analysis.
- *Statistical analysis.* Separate two-factor analysis of variance (ANOVA) was used to analyze the data obtained for number of avoidances for learning (1st and 2nd training day) and memory test (24 h after the 2nd training day) between subject factors: substances (three levels: HU-210, SR 141716A, and saline) and side of injection (two levels: left and right). ANOVA data were further analyzed by *SNK* test for *post hoc* group comparisons where appropriate.

RESULTS

Effects of HU 210 and SR 141716A microinjected unilaterally into CA1 hippocampal area of sham-operated rats

After the unilateral infusion of HU 210 and SR 141716A in the CA1 area, the two-factor ANOVA

showed a significant effect for the factor "drug" for the 1st training day ($F_{1,35}$ = 9.2129; P ≤ 0.0001) and the 2nd training day ($F_{2,35}$ = 28.3762; P ≤ 0.0001) and a nonsignificant effect for the factor "side" for the two training days ($F_{2,35}$ = 2.9629; P =NS) and ($F_{1,35}$ = 0.9278; P =NS). For the memory test, there was a significant effect for the factors "drug" ($F_{2,35}$ = 4.7107; P ≤ 0.05) and "side" ($F_{1,35}$ = 62.11957; P ≤ 0.0001) and for the interaction "drug" X " side" ($F_{2,\ 35}$ = 4.9053, P ≤ 0.02).

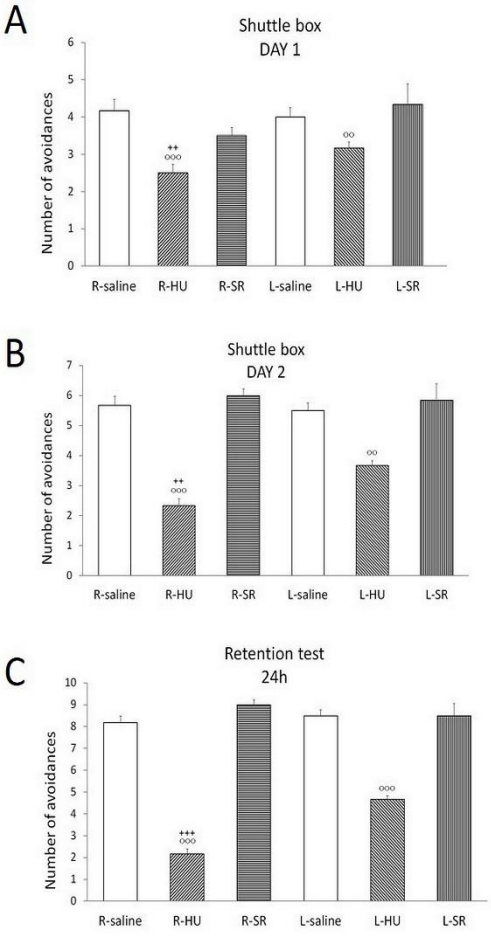

Fig. 1 (A, B, C). Effects of HU-210 (5 µg/0.1 µl) or SR 141716A (3 µg/0.1 µl) microinjected into the left (L) or right (R) CA1 hippocampal area of sham-operated rats on the number of avoidances (N Av). A) 1st training day; B) 2nd training day; C) retention test. $^{oo}P \leq 0{:}01$; $^{ooo}P \leq 0{:}001$ – comparisons of the N Av, following drug injections *vs*. resp. saline injections into the CA1 area. $^{++}P \leq 0.01$; $^{+++}P \leq 0{:}001$ – comparisons of the N Av, following injections into the right CA1 *vs.* left CA1 area. n = 6. Means (± S.E.M.) are presented.

HU 210 injected unilaterally into the CA1 area impaired the learning and memory processes of sham-operated rats. The *post hoc* SNK test showed that HU 210 microinjected into the right (R) or left (L) CA1 area reduced the number of avoidances compared to the corresponding saline-treated controls: on the 1st training day (R - P ≤ 0.001; L - P ≤ 0.01), 2nd training day (R - P ≤ 0.001; L - P ≤ 0.004) and the retention test (R - P ≤ 0.001; L -P ≤ 0.001) (Fig. 1. A, B, C).

Comparing the unilateral HU 210 infusions (left *vs.* right), the right-side infusions resulted in significantly fewer avoidances than left-side ones on the 1st and 2nd days (P ≤ 0.01; P ≤ 0.003) and at the memory test (P ≤ 0.001) (Fig. 1, A, B, C). *Post-hoc* test showed that SR141716A infused into the right or left CA1 area did not change the number of avoidances on the 1st and 2nd (P =NS) training day and at retention test (P =NS) compared to the respective controls treated with saline: on the 1st (P =NS) and 2nd (P =NS) training day and at retention test (P =NS) (Fig. 1. A, B, C).

Comparing the effects of SR141716A in the right *vs*. left CA1 hippocampal areas, no significant differences were found – on 1st (P = NS), 2nd (P = NS) days, and retention test (P = NS) (Fig. 1. A, B, C).

Effects of HU 210 and SR 141716A microinjected unilaterally into CA1 hippocampal area of olfactory bulbectomized (OBX) rats.

Two-factor ANOVA after unilateral microinjections of HU210 or SR141716A into the CA1 hippocampal area of OBX-rats showed, for the 1st training day, a significant effect for the factors "drug" ($F_{2,35}$ = 77.8666; P ≤ 0.0001), "side" ($F_{1,35}$ = 19.2666; P ≤ 0.0001) and significant interactions between " side" X "drug" ($F_{2,35}$ = 19.4666; P ≤ 0.0001); for the 2nd training day - a significant effect for the factors "drug" ($F_{2,35}$ = 15.9210; P ≤ 0.0001), "side" ($F_{1,35}$ = 58.9144; P ≤ 0.0001) and significant interactions "side" X "drug" " ($F_{2,35}$ = 20.5592; P ≤ 0.0001) and at the retention test – "drug" ($F_{2,35}$ = 146.6562; P ≤ 0.0001), "side" ($F_{1,35}$ = 40.500; P ≤ 0.0001) and "side" X "drug" ($F_{2,35}$ = 86.71875; P ≤ 0.0001).

HU 210 infused into the right CA1 area increased the number of avoidances in OBX-rats compared to both right-side saline-microinjected OBX rats and to sham-operated rats on 1st (resp. P ≤ 0.001; P ≤ 0.02) and 2nd (resp. P ≤ 0.0001; P ≤ 0.04) training day and at the memory test (resp. P ≤ 0.001; P ≤ 0.04). HU-210 microinjected into the left CA1 area increased the number of avoidances in OBX-rats compared to the left-side saline-microinjected OBX-rats on 1st (P ≤ 0.002) and 2nd (P ≤ 0.04) training day and at the retention test (P ≤ 0.002). Compared with sham-operated rats the number was lower on 1st (P ≤ 0.002) and 2nd (P ≤ 0.001) training day and at the

retention test ($P \leq 0.001$). HU 210 infused into the right-CA1 significantly increased the number of avoidances on the 1st ($P \leq 0.001$), 2nd ($P \leq 0.001$) training day and the retention test ($P \leq 0.0001$) as compared to the left-CA1 injections (Fig. 2. A, B, C).

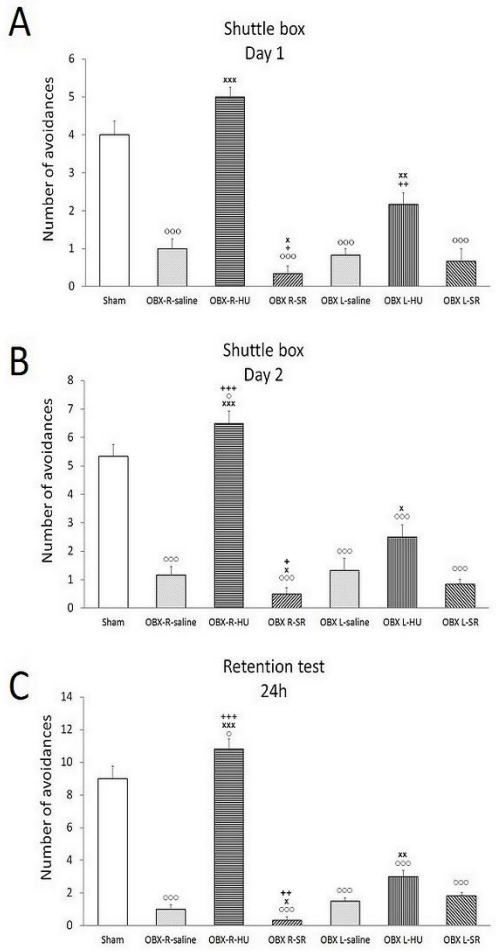

Fig. 2. (A, B, C). Effects of HU-210 (5 µg/0.1 µl) or SR 141716A (3 µg/0.1 µl) microinjected into the left (L) or right (R) CA1 hippocampal area of OBX rats on the number of avoidances (N Av). A) 1st training day; B) 2nd training day; C) retention test. $^{\circ}P \leq 0.05$; $^{\circ\circ}P \leq 0.01$; $^{\circ\circ\circ}P \leq 0.001$ – comparisons of the N AV, following drug injections *vs.* sham-operated rats; $^{x}P \leq 0{:}05$; $^{xx}P \leq 0{:}05$; $^{xxx}P \leq 0{:}001$ - comparisons of the N AV, following drug injections to OBX rats *vs.* OBX-saline injections into the respective CA1 area $^{+++}P \leq 0{:}001$ – comparisons of the N AV, following injections into the right CA1 area *vs.* left CA1 area in OBX rats. n = 6. Means (± S.E.M.) are presented.

SR141716A microinjected into the right CA1 area of OBX-rats decreased the number of avoidances compared to the corresponding OBX R-saline controls on 1st ($P \leq 0.03$), 2nd ($P \leq 0.05$) training day and at the memory test ($P \leq 0.03$); in the left CA1 area, it did not significantly change the number of avoidances compared to the OBX L-saline controls on 1st (P =NS), 2nd (P =NS) training day and at the retention test (P =NS). SR141716A-treated OBX rats showed fewer avoidances in all tests than the sham-operated rats ($P \leq 0.001$) (Fig.2. A, B, C). Comparing the effects of SR141716A into the right *vs.* left CA1 area of OBX-rats, it was found that SR141716A infused into the right side significantly decreased the number of avoidances on the 1st training day ($P \leq 0.05$) and at the retention test ($P \leq 0.05$) as compared to the left side (Fig.2. A, B, C)

DISCUSSION

Olfactory bulbectomy is an established animal model of depression. It triggers behavioral, immune, endocrine, neurochemical, etc. alterations in rodents, which resemble the ones observed in patients with depressive disorders. Behavioral disturbances, including memory deficit, are present two weeks after the surgical intervention [16]. The behavioral deficits occurring after OBX are associated with neurodegenerative changes and disrupted communication between different brain regions. The reduced spine density in the hippocampal CA1, CA3, and dentate gyrus most likely contributes to the impaired cognitive processes [17].

In a previous work we reported that CB receptor agonist HU 210, administered *i.c.v.*, deteriorates the learning and memory of rats and ameliorates the memory deficits of OBX rats. In contrast, SR 141716A had opposite effects on the rat avoidance performance [18]. This study extends our understanding of the learning and memory effects of cannabinoid ligands (HU 210 and SR 141716A) applied to the brain in rats with a model of depression (OBX). We have chosen the hippocampus because it is known to be a brain structure involved in learning and memory processes. Additionally, it is characterized by a high concentration of cannabinoid receptors. CB_1 receptors are expressed at a high density in the CA1, CA3 regions and dentate gyrus [19]. CB_2 receptors have low physiological expression, but it is enhanced in certain pathological conditions (e.g., neurodegenerative diseases, brain injuries, etc.).

In the present study, HU 210 microinjected unilaterally, into the left or right CA1 hippocampal area deteriorated learning and memory of sham-operated rats, tested in an active avoidance paradigm and the effect was more pronounced on the right side. The unilateral infusions of SR 141716A did not significantly affect the rat's performance. Our data contradict the findings of Wise et al. [20], who reported memory impairment following

intrahippocampal administration of the CB1 receptor antagonist SR 141716A.

Previously we have demonstrated that OBX impairs rat performance in both active and passive avoidance tasks [14]. We have also observed asymmetric learning and memory impairing effects after angiotensin 1 (AT1) receptor stimulation in the left amygdala of OBX rats, while inhibition of AT1 receptors in the left amygdala improved these processes and prevented memory deficits in OBX model of depression [21]. In the present study, the acute administration of HU 210 in the right CA1 area reversed the OBX-induced learning and memory deficits in the two-way active avoidance task, while microinjection into the left CA1 area only attenuated the acquisition and retention deficits. SR 141716A further impaired the rat's performance and its suppressive effect was present only on the right side. We hypothesize that the learning and memory-improving effects of HU 210 could be due to a modulatory influence on the altered activity of some neurotransmitter systems (GABA, 5-HT, Ach, etc.) which have been reported following olfactory bulbectomy. Following OBX, a significant reduction of long-term potentiation (LTP) in the hippocampal CA1 region is also observed [22]. It is well known that CB_1R agonists impair cognition and prevent LTP of synaptic transmission. A recent work demonstrated that CB_1R activation may affect CA1 LTP in an opposing way, depending on the strength of LTP induction and magnitude. It was shown that both a CB_1R inverse agonist, AM251, and a CB_1R antagonist Rimonabant lead to a facilitation of weakly induced LTP but to an inhibition of strongly induced LTP [23]. The opposing effects of cannabinoid ligands in sham-operated and OVX rats may also be related to the dual influence of endocannabinoids on LTP.

This study is the first to provide information on a positive effect of HU 210 on the acquisition and retention of active avoidance learning when injected into the CA1 hippocampal area in OBX rats. An important finding is that the cannabinoid ligands exerted an asymmetric effect on learning and memory. The differential responses to the unilateral (left or right) drug administration in OBX rats suggest an asymmetric distribution of CB1 receptors in the left and right CA1 areas. There is evidence for functional asymmetries in LTP, showing that exposure to a novel environment early in life enhances LTP selectively in the right CA1 area of adult rats, whereas the left hippocampus shows no differences [24]. Considering the above data, we can assume, albeit speculatively, that the CB receptor stimulation is most likely involved in the modulation of excitatory and inhibitory synaptic transmission, and hence in hippocampal LTP.

Our data on the effects of cannabinoid ligands infused unilaterally into the CA1 area of OBX rats are original and suggest that CB1 receptors are probably involved in the development of the depressive state.

CONCLUSION

The present study is the first to provide information on the modulatory effect of cannabinoid receptor ligands microinjected into the hippocampal CA1 area on the learning and memory of olfactory bulbectomized rats. It is demonstrated that stimulation of hippocampal CB receptors in a rat depression model (olfactory bulbectomy) improves rat performance in the active avoidance task. We observed also asymmetrical effects of cannabinoid ligands on learning and memory, suggesting a differential distribution of CB receptors in the brain hemispheres. The asymmetrical distribution of CB receptors in the left and right CA1 hippocampal area could contribute to the asymmetry in the cognitive effects of the CB receptor ligands.

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