



CB₁-RECEPTOR ANTAGONIST SR-141716A MODULATES LOCOMOTOR ACTIVITY IN OBX-RATS

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ABSTRACT

Dysfunction of the endocannabinoid system has been related to depressive-like behavior. In our study, bilateral olfactory bulbectomy (OBX) was used as an animal model of depression. We evaluated the effect of the selective CB₁-antagonist SR-141716A (Rimonabant) on the exploratory and locomotor activity of OBX-rats. Rimonabant was administered intragastrically for 14 days to OBX-rats, divided into 3 experimental groups, where the drug was given before; immediately (1-14 days) after; or 14 days (14-28 day) after OBX. Exploratory and locomotor activity of OBX-rats was tested in an Opto-Varimex apparatus. SR-141716A, administered subchronically, intragastrically exerted locomotor stimulating effects in OBX- and sham-operated rats, while the exploratory activity was not affected. The time interval for the drug administration is of significance for the manifestation of the effects on locomotor activity in OBX-rats. SR-141716A applied 14 days before OBX or after the development of a depressive-like state (14-28 days after OBX), but not immediately after OBX (1-14 days) aggravated the OBX-induced hyperlocomotor state.

Keywords: Cannabinoid receptor, olfactory bulbectomy, exploratory, locomotor, rat,

INTRODUCTION

The endogenous cannabinoid system (ECS) plays an important modulatory role in the central nervous system. ECS consists of endocannabinoids, cannabinoid receptors and the enzymes for the endocannabinoid synthesis and degradation. The major endogenous cannabinoids are N-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol. There are two cannabinoid receptors (CB₁ and CB₂), which belong to the superfamily of G-protein-coupled receptors. Cannabinoid receptors are widely distributed in the CNS, where the CB₁-receptor is the most abundant type. The receptors are located at both presynaptic terminals and postsynaptic compartments of neurons and on astrocytes, restricting their function to the sites of synaptic activity [1].

Bilateral olfactory bulbectomy (OBX) is an experimental model of depression, which produces in rodents a syndrome with behavioral, neurochemical, and structural abnormalities similar to that in patients with depressive disorder. Increased exploratory and locomotor activities of rodents in response to stress from exposure to new open space are major abnormalities, part of the bulbectomy-induced behavioral syndrome [2]. Evidence is provided that CB₁-receptors (CB₁R) are involved in the development of depression [3, 4]. However, studies have yet yielded conflicting results in different models of depression. Data on the behavioral effects of CB₁R-antagonists in animal models are also contradictory. SR-141716A is a selective antagonist of central CB₁R, which also has properties of inverse agonist [5]. Our previous research demonstrated that SR-141716A exerted opposite effects upon different routes of administration on the memory of OBX-rats, as well as a stimulatory effect on the locomotor activity of OBX-rats upon i.c.v.-administration [6, 7].

In the present experiments, the CB₁-antagonist was administered subchronically, intragastrically to OBX-rats, and its effects on the exploratory and locomotor activity were assessed. The aim of our study was twofold: 1/to evaluate the role of CB₁-receptor in the motor disturbances of OBX-rats; and 2/ to estimate the impact of different time intervals for drug administration on the behavioral effects.

MATERIALS AND METHODS

Bilateral olfactory bulbectomy (OBX) was performed according to the method described by Kelly et al. (1997) [2]. Rimonabant (SR-141716A, Sanofi) was administered by an intragastric tube at a dose of 3 mg/kg for 14 days to OBX- and sham-operated rats. Locomotor activity was assessed in an Opto Varimex apparatus. The apparatus recorded the number of photo beam interruptions during animal movements in the experimental chamber. The selective counting of the number of horizontal and vertical movements is presented in arbitrary units (AU). The information was recorded automatically for a 5-minute period of observation.

Male Wistar rats, treated with Rimonabant (RIM) or saline were divided into 7 groups: 1) Sham-RIM; 2) 14d RIM, OBX (RIM administered before OBX, test performed 14 days after OBX); 3) OBX, 1-14d RIM (RIM administered immediately after OBX (1-14 days), test performed 14 days after OBX); 4) OBX, 14-28d RIM (RIM administered after the development of a depressive-like state (14-28 day), test performed 28 days after OBX); 5) Sham-saline (sham-operated); 6) OBX14d-saline (OBX-rats, test performed 14 days after OBX); 7) OBX28d-saline (OBX-rats, test performed 28 days after OBX).

RESULTS

Two-way ANOVA with repeated measurements was used for the analysis of the number of horizontal and vertical movements for each minute for a 5-minute observation period. (Fig 1. A, B).

The factors were “drug” with 7 levels (sham-RIM; sham-saline; OBX14-saline; 14d RIM, OBX; OBX, 1-14d RIM; OBX28-saline; OBX, 14-28d RIM) and “time” with 5 levels (1, 2, 3, 4, 5 min). One-way ANOVA was used to analyze the total number of horizontal and vertical movements. Data obtained from ANOVA were analyzed by the post-hoc Student-Newman-Keuls (SNK) test (Fig 2, A, B).

Fig 1. a, b. Effect of Rimonabant (SR-141716A) on the number of movements in OBX-rats (horizontal movements - A and vertical movements - B) on each minute in a 5-minute period of observation

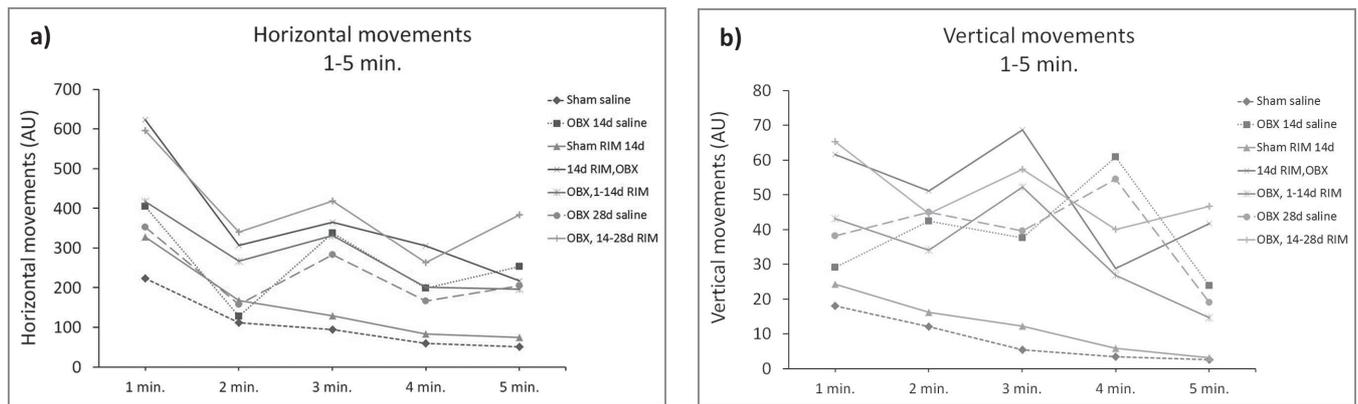
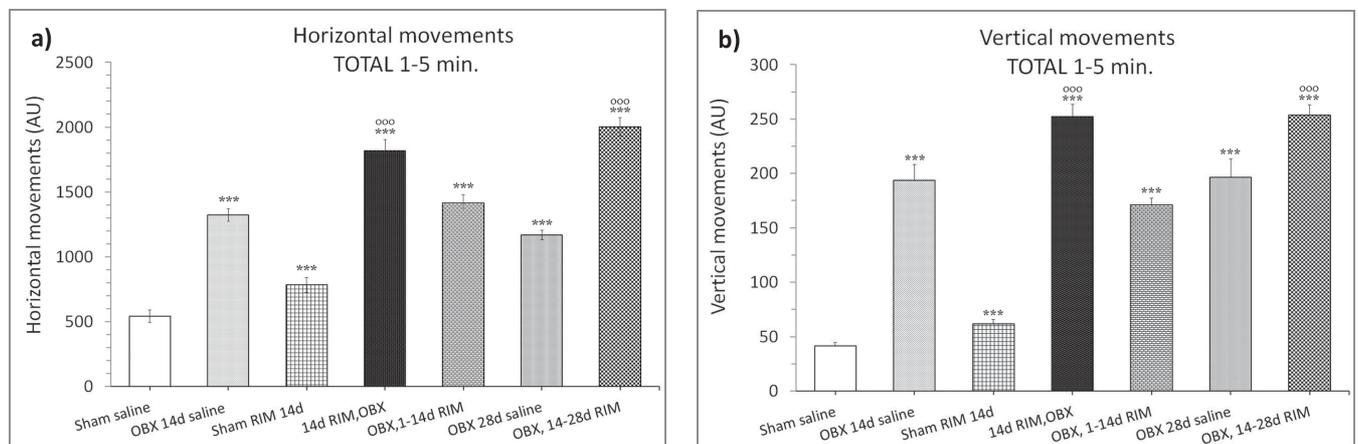


Fig 2, a, b. Effect of Rimonabant (SR-141716A) on the total number of horizontal movements (A) and vertical movements (B) in OBX-rats. *** $P \leq 0.001$ - comparison with sham-operated controls; 000P ≤ 0.001 - comparison with OBX-controls.



ANOVA for the number of movements in OBX-rats demonstrated significance for the factors “bulbectomy” ($F_{1,79} = 131.0274$; $P \leq 0.0001$) (horizontal), ($F_{1,79} = 127.3371$; $P \leq 0.0001$) (vertical), and “time” ($F_{4,79} = 28.0172$; $P \leq 0.0001$) (horizontal), ($F_{4,79} = 5.996$; $P \leq 0.0001$) (vertical), as well as a significant interaction between the factors ($F_{4,79} = 8.1899$; $P \leq 0.0001$) (horizontal), ($F_{4,79} = 8.6202$; $P \leq 0.0001$) (vertical). One-way ANOVA for the total number of movements showed significance for the “bulbectomy” factor for horizontal ($F_{1,15} = 126.1964$; $P \leq 0.0001$) and ($F_{1,15} = 103.7199$;

$P \leq 0.0001$) for vertical movements.

Two way ANOVA for the number of movements after RIM-administration to OBX-rats showed a significant effect on the “drug” factor ($F_{6,279} = 77.1675$; $P \leq 0.0001$) (horizontal) and ($F_{6,279} = 75.2631$; $P \leq 0.0001$) (vertical) movements ; “time” factor ($F_{4,279} = 95.4984$; $P \leq 0.0001$) – (horizontal) and ($F_{4,279} = 19.9945$; $P \leq 0.0001$) (vertical) movements ; as well as a significant interaction between the factors “drug” \times “time” ($F_{24,279} = 3.4967$; $P \leq 0.0001$) and ($F_{24,279} = 5.8580$; $P \leq 0.0001$) respectively (Fig 1, A, B).

One way ANOVA for the total number of movements showed significance for the “drug” factor ($F_{6,55} = 73.2426$; $P \leq 0.0001$) (horizontal) and ($F_{6,55} = 65.3767$; $P \leq 0.0001$) (vertical) movements (Fig 2, A, B).

OBX-rats demonstrated the specific pattern of abnormal motor activity, induced by the bulbectomy: hyperlocomotion and impaired exploration of the new area. The total number of both horizontal and vertical movements was increased, as well as the number of movements at 1st, 3rd, 4th and 5th minute as compared to the sham-operated controls (Fig 1. A, B, 2. A, B).

The administration of RIM in the three time intervals (before OBX, immediately after OBX, and on the background of developed depressive-like state, after day 14 of bulbectomy) did not show a significant effect on the impaired exploratory activity of OBX-rats (Fig 1. A, B).

However, the total number of horizontal and vertical movements in groups 14d RIM, OBX and OBX, 14-28d RIM was increased significantly ($P \leq 0.001$) as compared to the OBX-controls, while no changes were observed in the OBX, 1-14d RIM group (Fig 2. A, B). RIM did not affect the exploratory activity of sham-operated rats, demonstrated by a gradual decrease of movements during the 5 min period of observation (Fig 1. A, B).

However, locomotor activity was higher as compared to the saline-treated controls (total number of both horizontal and vertical movements was increased, $P \leq 0.001$) (Fig 2, A, B).

DISCUSSION

Hyperlocomotion of rodents in response to stress from exposure to new open space is a major component of the bulbectomy-induced behavioral syndrome [2]. Literature data indicate that the behavioral changes in OBX-rats appear about 14 days after the surgical removal of the olfactory bulbs. RIM, applied 14 days before OBX (14d RIM, OBX) or 14 days after the development of depressive-like state (OBX, 14-28d RIM), increased the number of horizontal and vertical movements (for each minute, as well the total number for a 5 min period of observation), compared to the OBX-controls. However, the treatment with RIM immediately after bulbectomy (OBX, 1-14d RIM) did not produce any significant effects on the number of movements compared to the OBX-controls. In addition, the intragastric administration of Rimonabant at the three time intervals did not affect the impaired exploratory activity of OBX-rats in the open space. The inability of OBX-rats to adapt to a new environment has been evidenced by the lack of gradual reduction of movements during the exploration. Our results are in accord with the findings that the ECS-dysregulation contributes to the hyperlocomotor response after bulbectomy [8, 9, 10]. According to Eisenstein et al., the ventral striatum (a brain region deafferented by OBX) is specifically related to the hyper motility of OBX-animals [9]. There are reports that the density of CB1-receptors in the prefrontal cortex and amygdala of OBX-rats is increased, and this

was associated with an up-regulation of CB1R-protein expression. It was shown that a single-dose CB1-agonist Δ^9 -THC (5 mg/kg, i.p) normalized motor activity in OBX-rats, while the 14-day administration of fluoxetine normalized the hyperactivity in rats and restored CB1R-density in the prefrontal cortex [8].

In general, the dose of the drug, route of administration, experimental design, and even the emotional state of the animal can modify the effects exhibited by the cannabinoid ligands. Previously we have demonstrated that the 7 day i.c.v.-administration of SR-141716A (3 mg/1 μ l) aggravated the OBX-induced hyperlocomotor state [6]. Based on the results from both previous and present studies, we conclude that the CB1-antagonist SR-141716A, upon different routes of administration, produced the same stimulatory effect on the locomotion of OBX-rats, tested in an Opto-Varimex apparatus (small arena).

Δ^9 -THC induced hypoactivity is a well-known pharmacological effect of cannabinoids [11]. Using CB1- and CB2-knockout mice, it was demonstrated that CB1- and CB2-receptor mechanisms underlie Δ^9 -THC-induced hypolocomotion [12]. Reports reveal a dose-dependent effects of cannabinoids on motor activity in experimental animals. For example, high doses of cannabinoids impair the expression of novelty-induced behavior, while low doses disrupt behavioral habituation resulting in increased motor and exploratory activity [13]. The blockade of CB1-receptors is also associated with contradictory effects on motor activity in experimental animals. Studies have shown that SR-141716A (i.p.) does not affect the decreased locomotor activity induced by CB1-receptor agonists, increases locomotor activity or inhibits it [14, 15, 16]. Our results support the findings of a study where SR-141716 (i.p.) produced a significant increase in locomotor activity in mice. The authors also provided evidence that the stimulatory effect was not a result of SR-141716A- inverse agonist activity at the CB1-receptor or of disinhibition of an endogenous tone [17]. The present findings of locomotor effects of RIM in OBX- and sham-operated rats during the 5-minute observation period are not in accord with the reports, where CB1-receptor blockade with AM251 or Rimonabant (1mg/kg, i.p.) in OBX-rats, increased locomotor activity during the habituation phase only (3-30 min), but not in the novelty phase, while in sham-operated rats, AM251 increased, Rimonabant decreased, distance traveled [10].

In our present work, we also aimed at evaluating the role of time interval for the manifestation of the drug effects of the locomotor and exploratory activity of OBX-rats. An important and unexpected finding was that only upon administration in the period immediately after the OBX-procedure, Rimonabant did not interfere with the development of motor disturbances. Exploratory activity, however, was not affected by the treatment during the three time intervals and remained impaired. The analysis of the results leads us to the conclusion that the effects

of the CB1-antagonist, administered 14 days before and 14-28 days after OBX on the exploratory and motor activity, are not directly related and that the drug specifically aggravated the OBX-induced hypermotility.

Evidence is provided that CB1-receptors are involved in the development of depression [3, 4]. Genetically modified CB1-receptor-deficient mice, which exhibit depressive behavior, have been proposed as an appropriate model to evaluate depressive-like disorders in animals [18]. There are some reports about an antidepressant activity of SR-141716. For example, subchronic administration of Rimonabant (4-8 mg/kg, i.p.) significantly decreased the duration of immobility time in forced swim test (FST) and in tail suspension test, without affecting the baseline locomotion, and reversed the hyperactivity exhibited by OBX-rats in an open field test [19]. A single administration of Rimonabant (10 mg/kg, p.o) reduced the immobility time of the FST to results similar to those of antidepressants, but a similar effect was absent in 14-day

treatment, which was associated with reduced neurogenesis in the hippocampus [20]. In the present study, we cannot interpret the aggravation of the OBX-induced hyperlocomotion after subchronic RIM-administration 14 days before and 14-28 days after OBX as a prodepressive-like effect, as far as the drug also increased the movements in the sham-operated rats.

CONCLUSIONS

SR-141716A, a selective CB1-receptor antagonist, administered subchronically intragastrically, exerts locomotor stimulating effects in OBX- and sham-operated rats, while the exploratory activity is not affected. The time interval for the drug administration is of significance for the manifestation of the effects on locomotor activity in OBX-rats. SR-141716A applied 14 days before OBX or after the development of a depressive-like state (14-28 days after OBX), but not immediately after OBX (1-14 days) aggravated the OBX-induced hyperlocomotor state.

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