CANNABIDIOL IMPROVES LEARNING AND MEMORY AND EXERTS ANXIOLYTIC EFFECT IN EXPERIMENTAL STUDY

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Abstract:

Cannabidiol (CBD) is a non-psychotomimetic constituent of Cannabis sativa. Experimental evidence indicates that CBD possesses antioxidant, anxiolytic and neuroprotective effects. The aim of the present study was to examine the effect of cannabidiol on learning and memory in scopolamine-induced memory impairment and its anxiolytic effect in a model of chronic stress. Materials and methods: Male Wistar rats were used in the experiments (n=8). For studying the anxiolytic effect animals were exposed to a chronic mild stress for 7 weeks. After that period the anxiolytic effect was evaluated using Vogel conflict test. The effect of CBD on learning and memory was examined in a model of memory impairment - scopolamine 1 mg/kg bw was applied intraperitoneally. Memory and learning were evaluated using step-down passive avoidance test. For each of the experiments animals were divided into five groups – control with olive oil, control with olive oil and chronic stress or memory impairment respectively, CBD 2,5; 5 and 10 mg/kg bw. The statistical analyses were performed using ANOVA followed by Tukey's multiple range test. Results: In Vogel conflic test animals treated with CBD 5 and 10 mg/kg significantly increased number of shock licks compared to the control group exposed to chronic stress. In the step-down passive avoidance test CBD 5 mg/kg bw significantly increased latency time on the short- and long-memory retention test compared to the control group with memory impairment. Conclusion: The results from our study suggest that CBD could improve learning and memory and exerts anxiolytic effect.

Key words: *Cannabidiol, anxiolytic, passive avoidance, Vogel conflict test*

Introduction

Cannabis sativa has been used in medicine since ancient times. In traditional Chinese medicine cannabis was applied for the treatment of asthma, malaria and gout and in India for convulsions, neuralgias and migraines [1]. The use of cannabis became very popular in Europe and USA in the nineteenth century. Cannabis tincture (ethanolic extracts of cannabis) was used to treat various disorders such as convulsions in infants, cholera, tetanus and rabies [2]. However, in the first half of the twentieth century these practices were abolished owing to an inability to prepare standardised cannabis preparations, which resulted in the risk of producing over- or under-dosed formulations [3].

Cannabis sativa contains over 100 compounds. Among them Δ9-tetrahydrocannabinol (THC) is considered the main component responsible for the psychoactive effects of the plant. Cannabidiol (CBD) is the main nonpsychotropic phytocannabinoid constituent of Cannabis sativa, corresponding to about 40% of the plant extract [4]. CBD was first isolated from Cannabis sativa in 1940 by Roger Adams, but its structure was not completely elucidated until 1963. THC was considered the "active" principle of Cannabis and research then focused primarily on it to the virtual exclusion of CBD [5].

In the last decades CBD has attracted researchers attention due to its wide therapeutic properties across a range of neuropsychiatric disorders - antipsychotic, analgesic, anticonvulsant, antiemetic, anti-inflammatory, anxiolytic activity [6, 7]. Several preclinical studies showed improved working memory, object and social recognition, spatial learning and memory in several neurodegenerative models including the model of Alzheimer's disease

(AD), in inflammation and in neurological deficits, following both chronic or acute CBD application [8]. CBD has been found in vitro to be neuroprotective, to prevent hippocampal and cortical neurodegeneration, to have anti-inflammatory, antioxidant and antiapoptotic properties, diminish tau hyperphosphorylation and to regulate microglial cell migration [4, 9]. Futhermore, studies have shown thar CBD could have protective effect against ischemic injuries through reduction of infarction site [10].

The aim of the present study was to examine the effect of cannabidiol on learning and memory in scopolamine-induced memory impairment and its anxiolytic effect in a model of chronic stress.

Material and methods:

Ethical statement

The experiment has been approved by Animal Health and Welfare Directorate of the Bulgarian Food Safety Agency, permit № 257/2019 and by the Ethic Committee at Medical University of Plovdiv.

Drugs: cannabidiol - organic CBD, Nature's Way; scopolamide purchased from Sigma-Aldrich

Model of impaired memory

Male Wistar rats (200±20 g bw) were used in the study. Animals were kept under standard laboratory conditions - 12 h light/dark cycle; food and water ad libitum. Animals were divided into five groups (n=8) and were treated for 14 days as follows: I group – control olive oil per os; II group – control olive oil per os with memory impairment; III group – CBD 2,5 mg/kg b.w. per os; IV group – CBD 5 mg/kg b.w. per os; V group – CBD 10 mg/kg b.w. per os

After two-week period learning ability and memory retention were evaluated using one-way step down inhibitory "passive" avoidance test (Ugo Basile, Italy). Memory impairment was induced on the days of the experiment 30 minutes after administration of the substances by applying scopolamine (SCP) 1 mg/kg bw intraperitoneally to groups 2 to 5. Learning and memory were evaluated 30 min after inducing memory impairment. The first day of the experiment was the training session, on the second day short-term memory impairment was studies. Long-term memory retention test was performed on the day 7. Each day included 2 sessions with a 60-minute interval. The animal was placed on a plastic platform which vibrates vertically after switching on the apparatus. When the rat stepped down off it on 3 or 4 paws, electrostimulation (0.4 mA) was delivered by the cage grid floor outside the platform. Cutt-off time for each session was 60 sec.

Model of chronic stress

Male Wistar rats (200±20 g bw) were used in the study. Animals were divided into five groups (n=8) as follows: I group – control olive oil per os; II group – control olive oil per os exposed to chronic stress; III group – CBD 2,5 mg/kg b.w. per os; IV group – CBD 5 mg/kg b.w. per os; V group – CBD 10 mg/kg b.w. per os

Animals from groups 2-5 were exposed to a model of chronic mild stress for 8 weeks which consisted of the following stressors:

- cage tilt 45° for 24 hours,
- damp bedding – 200 ml water in 100 g sawdust for 24 hours,
- flashing light (60 flashes/minute) for 3 hours,
- predator sounds (recording of an adult cat) for 15 minutes,
- food deprivation for 24 hours, followed by restricted access of food,
- water deprivation for 24 hours, followed by empty water bottle exposure for 1 hour.

The animals were exposed to one stressor at a time. Each stressor was applied randomly in order to avoid adaptation. After the 8 week period the axiolytic effect of CBD was studied using Vogel conflict test (Ugo Basile, Italy). The test was performed in a box with a stainless grid floor. The metallic spout of a drinking bottle containing water projected into the box.

Animals were deprived of water for 48 hours before the experiment. After the first 24 hours animals were allowed to drink freely for 3 min in the test cage in order to find the drinking bottle spout. On the experimental day the contact of the animal with the spout and the grid floor closed an electrical circuit controlled by a sensor, which produced electric shock. The sensor recorded the total number of licks and punished (shock) licks delivered during 3-min period (alternation of 30 sec of electric shock and 30 sec without shock).

Statistics

Statistical analyses were performed using IMB SPSS software 19.0. Mean values ± SEM were calculated. Results were considered significant at $P < 0.05$. ANOVA was used to compare groups' parameters followed by Tuckey's multiple comparison test.

Results

Effect of CBD on one-way step-down inhibitory avoidance test in rats with scopolamine-induced memory impairment: Animals treated with scopolmine decreased latency of reactions on the 2^{nd} and 7^{th} day of the experiment compaired to the olive oil control group for the respective day ($p<0,05$). Rats treated with CBD 5 mg/kg and scopolamine significantly increased latency time on the second day of the experiment (short-term memory retention test) compared to the animals receiving olive oil and scopolamine ($p<0,05$), as well as compared to the control group without memory impairment ($p<0,05$). In the long-term memory retention test all studied doses showed significant increase in this parameter in comparison to both control groups ($p<0,05$) (fig.1).

Effect of CBD on Vogel conflict test in rats subjected to chromic mild stress: In Vogel conflict test animals treated with CBD 5 and 10 mg/kg and subjected to chronic mild stress significantly increased number of punished (shock) licks (fig.2) and number of total licks (fig.3) compared to both control groups ($p<0,05$).

Discussion

In our study all doses of CBD improved long-term memory in the step-down inhibitory (passive) avoidance test in a model of scopolamine-induced memory impairment. Numerous sources have shown that scopolamine is capable of blocking cholinergic neurotransmission. Acetylcholine (Ach) is the central neurotransmitter that mainly relates to learning and memory processes. This ability gave grounds for the extensive use of scopolamine to induce AD-like pathology in vivo and in vitro. Scopolamine can compromise the processes of learning acquisition and consolidation, significantly reduce Ach activities, and can raise oxidative stress in the hippocampus and prefrontal cortex in mice [11].

One-trial step-down inhibitory (passive) avoidance in rodents is hippocampus-dependent learning test and has long been a preferred model for biochemical and pharmacological studies of memory and induces long-term potentiation (LTP) in CA1 region of the hippocampus [12, 13]. Barichelo et al have found that prolonged treatment with cannabidiol, 10 mg/kg, prevented memory impairment in rats with pneumococcal meningitis in the inhibitory avoidance task [14]. The results from our study confirm these findings, demonstrating the pro-cognitive effect of CBD.

The Vogel conflict test measures the ability of drugs to release the drinking behaviour of water-deprived rodents exposed to a mild aversive stimulus (punishment). This model has been widely employed in the evaluation of potential anxiolytic agents [15]. Moreira et al have shown

that CBD induces an anxiolytic-like effect in the Vogel conflict test, similar to the effect of diazepam, which are in concordance with our results [16]. In our study CBD 5 and 10 mg/kg bw increased number of shock licks in the Vogel conflict test, confirming the anxiolytic effect of cannabidiol.

The improvement of cognitive function and the anxiolytic effect of CBD can be explained by CBD's multiple mechanisms of action.

The endocannabinoid (eCB) system plays a crucial role in the regulation of emotional behavior and is essential for synaptic processes that regulate learning and emotional responses, specifically those related to potentially traumatic experiences [17]. eCB signaling is distributed throughout the CNS and peripheral tissues, modifying presynaptic release of both excitatory and inhibitory neurotransmitters. Cannabinoid type 1 (CB1) receptors are expressed by peripheral and central neurons, especially in the central areas that play key roles in anxiety and aversive learning - the amygdala, hippocampus and cerebral cortex [18]. CBD has minimal affinity for CB1 and CB2 receptors, but could indirectly activate CB1 receptors by increasing the availability of endogenous endocannabinoids. Anandamide (AEA) is an endogenous cannabinoid that acts as a partial agonist of CB1 receptors and is metabolized by the enzyme fatty acid amide hydrolase (FAAH). CBD has been reported to inhibit FAAH, thus increasing the availability of anandamide and causing activation of the endocannabinoid system. Studies in rodent models have shown that pharmacological activation of the eCB system through CB1-receptor agonists results in decreased behavioral response to aversive memories through the inhibition of memory reconsolidation and enhanced extinction [19].

Additionally it has been recently reported that systemic administration of CBD to mice dose-dependently increased the levels of Ach in the forebrain within hours. Therefore, there is evidence of association between cannabidiol, the cholinergic muscarinic neurotransmission system and cognitive function [20].

Another pharmacological mechanism involved in the anxiolytic and neuroprotective effects of CDB is facilitation of serotonin $5HT_{1A}$ receptor mediated neurotransmission in defense-related areas. While in vitro studies suggest that CBD acts as a direct 5-HT_{1A} receptor agonist, in vivo studies are more consistent with CBD being an allosteric modulator, or facilitator of 5-HT_{1A} signaling [21]. The 5-HT_{1A} receptor is a well-known anxiolytic target. Furthermore, studies have showed that the beneficial effects of CBD on cognitive and locomotor deficits, seen in a rat model of encephalopathy, are mediated by the $5HT_{1A}$ receptor [22].

In rodents, exposure to protocol of chronic stressors, induce alterations in dendritic remodeling and diminish adult hippocampal neurogenesis [23]. It has been reported that the chronic administration of a synthetic cannabinoid enhances neurogenesis in rats. Recent data has suggested that CBD not only could boost adult hippocampal neurogenesis, but additionaly could prevent the neurogenic disruption observed in a genetic murine model of AD through a peroxisome proliferator-activated receptor γ (PPARγ)-dependent mechanism. Regarding the hippocampus, repeated application of CBD could prevent the anxiogenic effect of chronic stress by facilitating hippocampal neurogenesis [23]. The results from our study confirm these finding since the test that we have chosen for studing the effect of CBD on cognition, one-way step-down inhibitory avoidance test, is dependent on hippocampal integrity.

Conclusion: The results from our study suggest that CBD could improve learning and memory and exerts anxiolytic effect.

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Fig. 1 Effect of cannabidiol on the latency time in step-down inhibitory (passive) avoidance test in rats with scopolamine-induced memory impairment
*p<0,05 versus the control group with scopolamine; 0p<0,05 versus the control group with olive oil

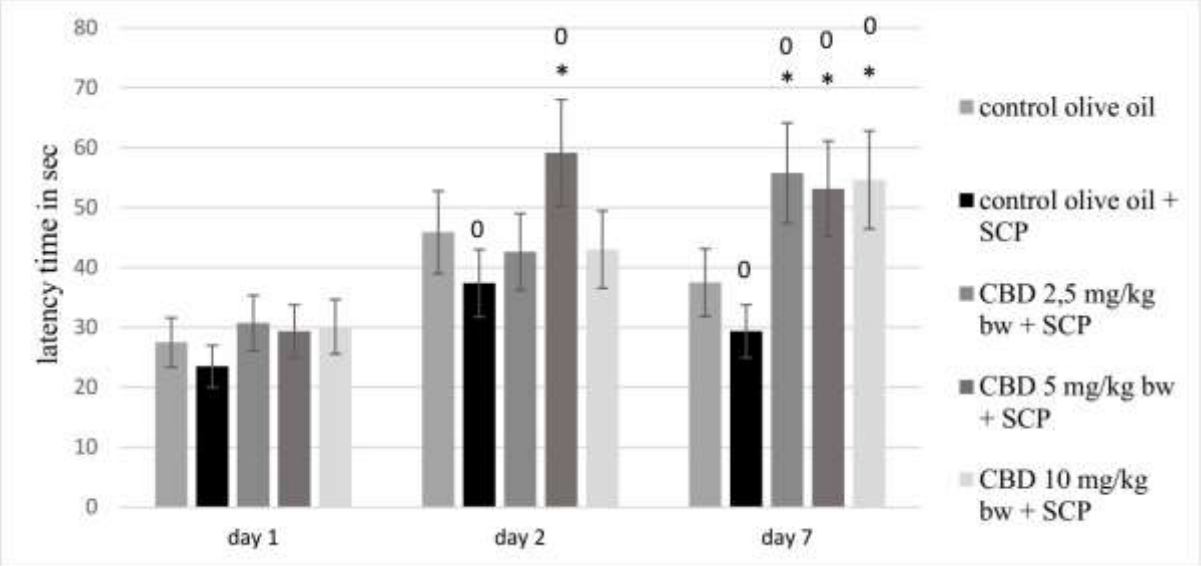
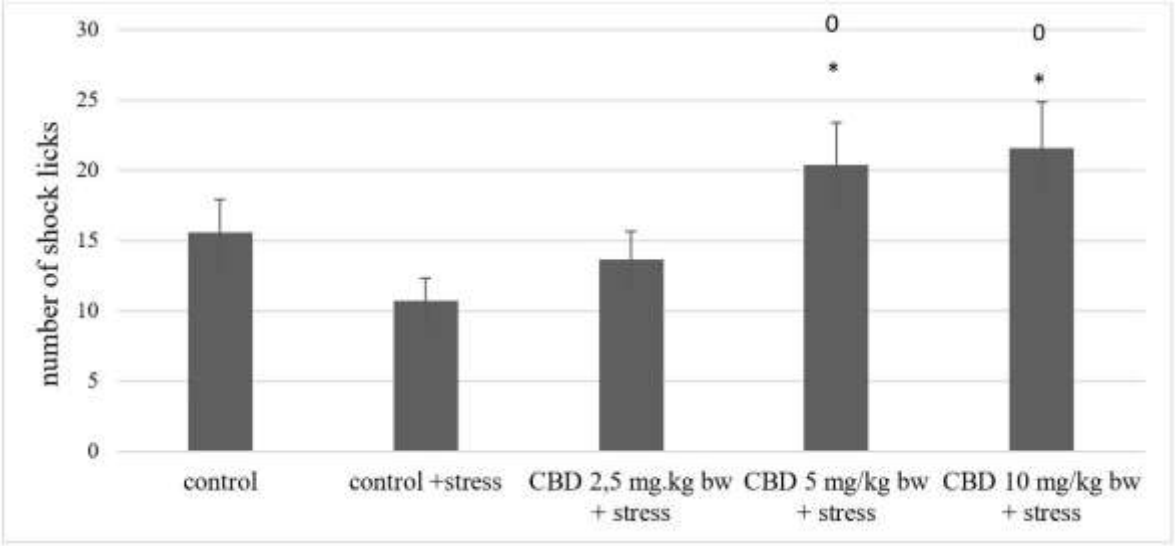


Fig. 2 Effect of cannabidiol on the number of shock licks in the Vogel conflict test in rats subjected to chronic mild stress



*p<0,05 versus the control group with stress; 0p<0,05 versus the control group with olive oil

Fig. 3 Effect of cannabidiol on the number of total licks in the Vogel conflict test in rats subjected to chronic mild stress

*p<0,05 versus the control group with stress; 0p<0,05 versus the control group with olive oil