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Impacts of cannabinoid receptor ligands on nicotine- and chronic mild stress-induced cognitive and depression-like effects in mice

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ABSTRACT

Taking into account the rather frequent concomitance of nicotine abuse and stress, we aimed to research memory- and depression-related effects of nicotine administration in combination with chronic mild unpredictable stress (CMUS) in mice and an involvement of the endocannabinoid system through CB1 and CB2 receptors. Mice were submitted to the CMUS for 4 weeks. Effects on depression-like behaviors and cognition, exerted by a combined administration of CB1, *i.e.*, Oleamide (2.5, 5.0 mg/kg), AM 251 (0.1, 0.25 mg/kg) and CB2, *i.e.*, JWH 133 (0.5, 2.0 mg/kg), AM 630 (0.25, 2.0 mg/kg) receptor ligands and nicotine (0.05, 0.1, 0.2 and 0.5 mg/kg), were then studied in stressed and unstressed mice by the forced swimming test and the passive avoidance paradigm, respectively. The results revealed that the CMUS-exposed mice exhibited depression-like behaviors and memory disturbances, while both effects were alleviated by nicotine. CB1 receptor ligands decreased antidepressive and cognitive (the latter for CB1 receptor antagonist only) effects of subchronic nicotine administration in stressed mice. CB1 and CB2 receptor antagonists exerted themselves some procognitive effects in those mice. Regarding the unstressed mice, CB1 and CB2 receptor ligands reversed the antidepressive effects of subchronic nicotine administration, while nicotine, in an ineffective dose, co-administered with CB2 receptor ligands, improved cognition. We confirmed the role of the two main subtypes of cannabinoid receptors, termed CB1 and CB2, on stress- and nicotine-related behavioral changes in mice. Our study has contributed to the understanding of the mechanisms involved in stress- and nicotine-induced disorders, such as anhedonia and memory disturbances.

1. Introduction

The experimental animal model of chronic mild unpredictable stress (CMUS) is broadly used and considered one of the best anhedonia models so far. It efficiently imitates unpredictable, intermittent exposure to stress, as well as the nature of mild stress experience in humans. It consists of repeated exposure to an array of varying and unpredictable mild stressors over a sustained period of time [1–4]. Rodents, subjected to prolonged exposure to mild stressors, demonstrated significantly reduced locomotor activity and decreased consumption of the rewarding, palatable foods, which may be interpreted as reduced sensitivity to reward [5,6]. Moreover, the applicability of this model to produce a state of anhedonia was supported by data demonstrating deficits in other measures of reward and hedonic impacts, such as conditioned place preference, brain stimulation reward and dopaminergic release in response to rewarding stimuli [6,7] reversed by an administration of antidepressant drugs [8].

It can be mentioned, in the context of our study, that certain scientific researches, including ours, indicate chronic stress to cause cognitive deficits and other mental and neurobiological disturbances [7,9,10]. One of the most significant, stress-related physiological changes is the activation of the hypothalamic–pituitary–adrenal (HPA) axis, associated with an excessive release of stress hormones, like cortisol, into the blood. In consequence, glucocorticoids bring damage to neurons of the hippocampus and prefrontal cortex, the two structures responsible for emotional reactions [10–13]. High blood glucocorticoid levels provoke physical harm of many dopaminergic, glutaminergic and serotoninergic neurons, may also suppress the process of neurogenesis and reduce hippocampus and prefrontal cortex volumes, which is characteristic of patients with depression [1,2].

Cannabinoids, as components of *Cannabis sativa* L, were discovered several thousand years ago. Today, they are mainly associated with their potential of addiction, although their therapeutic properties have for long been known and applied. The cannabinoid system is composed of endogenous agonists and their specific receptors, and also of proteins which control the levels of endocannabinoids in tissues [14]. CB1, the

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main cannabinoid receptors, are mostly localized presynaptically in the cellular membranes of the central (CNS) and peripheral nervous system neurons, and their activation inhibits the release of many neurotransmitters, including acetylcholine, noradrenaline, dopamine, serotonin, glutamate or γ-aminobutyric acid (GABA) and hormones. CB2 receptors are mainly found on immune system cells, especially on lymphocytes, macrophages and monocytes. Their activation inhibits release of the proinflammatory and increases anti-inflammatory cytokine [15]. However, CB2 receptors can also be found on the brain structures, such as the cerebellum and the hippocampus and in microglial cells [16,17]. It should be pointed out that, due to localization of CB receptors, the endocannabinoid system is strongly involved in the control of many emotion-related responses in the CNS, such as stress, anxiety, depressed mood and cognition [18–21].

The relationships between prolonged exposure to stress and the function of the endocannabinoid system is now the subject of many studies, since a controlled activation of the endocannabinoid system may open new, therapeutic perspectives, giving a chance to treat cognitive disturbances which accompany various neuropsychiatric, stress-related conditions [22]. From the point of view of the potential influence of the endocannabinoid system on the symptoms of affective disorders and considering that endocannabinoids play an important role in the physiology and behavioral expression of stress responses [19], it would thus seem important and justified to establish mutual interactions between endocannabinoid and other main neurotransmitter systems, including cholinergic, serotoninergic, noradrenergic and dopaminergic ones. It would also seem important, in the context of our experiments, to mention some significant interactions, which occur among stress, cannabinoid action and, additionally, nicotine effects [3,4]. Nicotine, as the main component of tobacco smoke, influences mood and emotional tension, also contributing to physical and psychological dependence. It has been suggested that stress plays a significant role not only in addiction genesis but also in abstinence maintenance [4]. For instance, it has been found that human exposure to stressors increases the number of smoked cigarettes, enhances the urge to smoke and expands the volume of inhaled tobacco smoke [8,23].

Taking into account the frequent concomitance of nicotine abuse and daily life stress situations, our research focused on the assessment of behavioral effects of acute and subchronic administration of nicotine in combination with CMUS and the ligands of cannabinoid CB1 and CB2 receptors in mice, based on our previous, fairly promising data [3,4]. Special emphasis was laid to investigate the influence of the CMUS procedure and nicotine on depression-like and cognitive effects, measured in the forced swim test (FST) and in the passive avoidance (PA) paradigm in male Swiss mice. The experiments were primarily focused on the complex involvement of the endocannabinoid system in the pathogenesis of stress- and nicotine-related depressive and cognitive behavioral changes. In total, this report discusses, among others, the mechanism of action of CB receptor ligands and their impact on stress-related behaviors, interactions between endocannabinoid and cholinergic systems and a possible therapeutic use of these compounds in stress-related responses, in which mainly the cholinergic system is implicated.

2. Materials and methods

2.1. Animals

The experiments were carried out on 2-month old naive male Swiss mice (Farm of Laboratory Animals, Warsaw, Poland) weighing 20–25 g at the beginning of the experiments. The animals were maintained under standard laboratory conditions (12 h light/dark cycle, room temperature 21 ± 1 °C) with free access to tap water and laboratory chow (Agropol, Pulawy, Poland) and were adapted to the laboratory conditions for at least one week. Each experimental group consisted of 8–12 animals. All experiments were conducted according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Council Directive for the Care and Use of Laboratory Animals of September 22, 2010 (2010/63/EU) and were approved by the local ethics committee (Permit Number: 37/2015).

2.2. Drugs

The following compounds were tested: (–) nicotine hydrogen tartrate (0.05, 0.1, 0.2 and 0.5 mg/kg, Sigma-Aldrich, St. Louis, MO, USA); CB receptor ligands, *i.e.*,

Oleamide (2.5 and 5.0 mg/kg, Sigma-Aldrich, St. Louis, MO, USA); a CB1 receptor agonist;

AM 251 (0.1 and 0.25 mg/kg, Tocris, Bristol, UK); a CB1 receptor antagonist;

JWH 133 (0.5 and 2.0 mg/kg, Tocris, Bristol, UK); a CB2 receptor agonist;

AM 630 (0.25 and 2.0 mg/kg, Tocris, Bristol, UK); a CB2 receptor antagonist.

Nicotine was dissolved in saline solution (0.9% NaCl), CB receptor ligands were suspended in a 1% solution of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) and also dissolved in saline solution. Nicotine was administered subcutaneously (s.c.) whereas CB receptor ligands were administered intraperitoneally (i.p.) at a volume of 10 ml/kg. Drug doses refer to the salt form. The pH of the nicotine solution was adjusted to 7.0. Fresh drug solutions were prepared on each day of experimentation. Control groups received saline injections of the same volume and *via* the same route of administration.

The dose and time interval for nicotine pretreatment and CB receptor ligands treatment were based on our previous experiments [3,4,19–22,24,25], literature data as well as preliminary studies. Doses of nicotine administered subchronically were slightly lower compared to its acute administration.

2.3. Behavioral experiments

2.3.1. CMUS procedure

The CMUS protocol was performed as described previously in our studies [3,4] and presented in Fig. 1. In brief, stressed mice were subjected to different kinds of mild stressors, which varied from day to day to make the stress procedure unpredictable. These stressors were randomly scheduled over a 1-week period and repeated throughout the 4 weeks (day 1–27) for 2 h daily. Unstressed mice, left undisturbed in their home cages, were exposed to behavioral tests, and not subjected to the CMUS procedure. 24 h after the end of the CMUS protocol, all independent groups of mice were exposed to one of the behavioral paradigms. Nicotine was administered acutely 30 min before the test (day 28) or subchronically (days 15–27) 30 min before the stressor to stressed as well as, to unstressed control mice - at the same time point. CB receptor ligands were also administered alone or with nicotine, 30 min before behavioral tests (day 28) to stressed and unstressed groups treated with nicotine or saline.

2.3.2. Forced swim test (FST)

The FST was described by Porsolt et al. [26]. In brief, each mouse was placed individually in a glass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C and was forced to swim for 6 min. The duration of immobility was recorded during the last 4 min of the test. A mouse was considered to be immobile when it stopped struggling and passively moved to remain floating and keep its head above water. Water was changed between trials.

2.3.3. Passive avoidance (PA)

The apparatus and procedure used was described in detail in a previous article [27]. The apparatus consisted of two-compartment