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Chronic and acute adenosine A_{2A} receptor blockade prevents long-term episodic memory disruption caused by acute cannabinoid CB_1 receptor activation

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ABSTRACT

Cannabinoid-mediated memory impairment is a concern in cannabinoid-based therapies. Caffeine exacerbates cannabinoid CB_1 receptor (CB_1R)-induced memory deficits through an adenosine A_1 receptor-mediated mechanism. We now evaluated how chronic or acute blockade of adenosine A_{2A} receptors ($A_{2A}Rs$) affects long-term episodic memory deficits induced by a single injection of a selective CB_1R agonist. Long-term episodic memory was assessed by the novel object recognition (NOR) test. Mice received an intraperitoneal (i.p.) injection of the CB_1/CB_2 receptor agonist WIN 55,212-2 (1 mg/kg) immediately after the NOR training, being tested for novelty recognition 24 h later. Anxiety levels were assessed by the Elevated Plus Maze test, immediately after the NOR. Mice were also tested for exploratory behaviour at the Open Field. For chronic $A_{2A}R$ blockade, KW-6002 (istradefylline) (3 mg/kg/day) was administered orally for 30 days; acute blockade of $A_{2A}Rs$ was assessed by i.p. injection of SCH 58261 (1 mg/kg) administered either together with WIN 55,212-2 or only 30 min before the NOR test phase. The involvement of CB_1Rs was assessed by using the CB_1R antagonist, AM251 (3 mg/kg, i.p.). WIN 55,212-2 caused a disruption in NOR, an action absent in mice also receiving AM251, KW-6002 or SCH 58261 during the encoding/consolidation phase; SCH 58251 was ineffective if present during retrieval only. No effects were detected in the Elevated Plus maze or Open Field Test. The finding that CB_1R-mediated memory disruption is prevented by antagonism of adenosine $A_{2A}Rs$, highlights a possibility to prevent cognitive side effects when therapeutic application of CB_1R drugs is desired.

1. Introduction

Cannabinoid research has a twofold interest: 1) to identify neuronal adaptation/dysfunction as a consequence of cannabinoid abuse and 2) to appraise the potential and eventual side effects of cannabinoid based therapies against several nervous system disorders, as it is the case of chronic pain (Carter et al., 2015), epilepsy (Maa and Figi, 2014) and several neurodegenerative diseases as Alzheimer's' Disease, Huntington Disease, Multiple Sclerosis, among others (de Lago et al., 2012; Fagan and Campbell, 2014; Fitzpatrick and Downer, 2016). Indeed, the brain cannabinoid system has been regarded as a new therapeutic frontier in brain repair (Fagan and Campbell, 2014; Maccarrone et al., 2014). A major concern in

Abbreviations: AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; A_1R, adenosine A_1 receptor; $A_{2A}R$, adenosine A_{2A} receptor; C57BL6/J, Black-Six mice; CBR, cannabinoid receptor; CB_1R, cannabinoid receptor 1; CB_2R, cannabinoid receptor 2; CZ, Open Field Test Central zone; DMSO, Dimethylsulfoxide; EPM, Elevated Plus Maze Test; GABA, Gamma-Aminobutyric acid(γ-Aminobutyric acid); I.P, intraperitoneal injection; IZ, Open-Field Test Intermediary zone; KW-6002, istradefylline; NOR, Novel Object Recognition Test; PZ, Open-field Test Peripheral zone; SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5 c]pyrimidine); VGCCs, Voltage-gated calcium channels; WIN55,212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthyl-methanone mesylate.

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the development of cannabinoid-based therapies is the memory and emotional dysfunction as 'on target' side effects (Copeland et al., 2013; Lovelace et al., 2015). Strategies that could prevent the behavioural consequences of cannabinoid intake, without affecting the neuroprotective actions of these substances, would be a breakthrough for further development of cannabinoid-based therapies.

Cannabinoids affect neuronal function by activating specific membrane located receptors, the cannabinoid receptor 1 (CB_1R) and the cannabinoid receptor 2 (CB_2R), which belong to the $G_{i/o}$ family of seven transmembrane G protein-coupled receptors, as well as other receptor types such as transient receptor potential (TRP) channels and peroxisome-proliferator-activated receptors (PPARs) (Akopian et al., 2009; O'Sullivan, 2016). CB_1Rs are the predominant receptor type in most neuronal cells and are those primarily responsible for the psychoactivity of exogenous cannabinoids and for the synaptic actions of endocannabinoids (Kano et al., 2009). In the adult brain, CB_1R are abundantly expressed throughout the brain, particularly in the hippocampus (Kano et al., 2009), a brain area mostly involved in consolidation of newly acquired information. One immediate consequence of cannabinoid intake is impairment of recent memory, reported in humans (Borgelt et al., 2013; Ranganathan and D'Souza, 2006) and documented in studies using laboratory animals (Clarke et al., 2008; Kano et al., 2009; Sousa et al., 2011). The hippocampus plays a predominant role in the memory disruptive effects induced by cannabinoid consumption (Wise et al., 2009).

In previous studies, we (Sousa et al., 2011) and others (Panlilio et al., 2012), observed that caffeine intake exacerbates memory impairment induced by cannabinoids. Caffeine is an antagonist of adenosine receptors being about equipotent for the two high affinity adenosine receptors, the A_1R and the $A_{2A}R$ (Sebastião and Ribeiro, 2009). $A_{2A}R$ is known to interact with CB_1Rs in brain areas where they are highly expressed, as the basal ganglia (Ferré et al., 2010; Martire et al., 2011; Tebano et al., 2012; Chiodi et al., 2016; Ferreira et al., 2015). Actions of caffeine upon $A_{2A}R$ often result in neuroprotection (Chen et al., 2001; Cunha et al., 2008; Rivera-Oliver and Díaz-Ríos, 2014). Prolonged intake of caffeine as well as of an $A_{2A}R$ antagonist, KW-6002 (istradefylline), was shown to revert long-lasting behaviour and synaptic impairments induced by chronic or unpredictable stress (Batalha et al., 2013; Kaster et al., 2015). In contrast, caffeine-induced exacerbation of memory impairments caused by cannabinoids results from its action upon A_1R (Sousa et al., 2011).

Considering the influence of adenosine upon synaptic plasticity phenomena (de Mendonça and Ribeiro, 1997; Dias et al., 2012) and that $A_{2A}Rs$ at the forebrain mostly act as metamodulators, regulating other modulatory systems (Sebastião and Ribeiro, 2009), including the influence of A_1R upon synaptic transmission (Cunha et al., 1994a; Lopes et al., 2002), the present work was designed to evaluate the influence of $A_{2A}Rs$ in memory dysfunction induced by cannabinoids. As a starting point, we used the novel object recognition (NOR) test, a memory test in which performance has been reported to be affected by CB_1R agonists (Clarke et al., 2008). Subsequently, we evaluated weather $A_{2A}R$ antagonists could influence that action. Remarkably, we found that chronic or acute blockade of $A_{2A}R$ abolishes the disruptive effect of a CB_1R agonist upon memory. This finding opens a window for the development of pharmacological strategies aiming to mitigate cognitive side effects of cannabinoid based therapies.

2. Materials and methods

2.1. Animals

Adult male 8–12 weeks old Black-Six (C57BL/6) mice (Charles River, Barcelona, Spain) were used. Animals were housed in a temperature and humidity regulated room with a 12-hour dark/light cycle, and free access to food and water. Experiments were performed during the light phase and around the same hour of the day. All experimentation followed the European Community Guidelines (Directive 2010/63/EU) and the Portuguese law (DL 113/2013) for Animal Care for Research Purposes, and have been approved by the "Instituto de Medicina Molecular" Internal Committee and the Portuguese Animal Ethics Committee –Direcção Geral de Veterinária). All efforts to reduce anxiety and stressful stimulus were taken into account. Animals were habituated to the presence of the investigator and to the experimental manipulation during a 5-day animal handling phase before testing. Five series of animals were used, as indicated in Fig. 1. Animals in each series were purchased, handled and tested together, being randomly allocated to the different drug groups. Drug effects were always taken from comparisons with controls within the same series. All animals were sacrificed by decapitation under deep anaesthesia within two days after the experiments have ceased.

2.2. Drugs

KW-6002 (istradefylline) was synthesized according to a published procedure (Hockemeyer et al., 2004). SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo [1,5 c]pyrimidine), was obtained from Sigma-Aldrich (MO,USA). WIN55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthyl-methanone mesylate), and AM251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) were obtained from Tocris Bioscience. WIN 55,212-2, AM251 and SCH 58261 were suspended in dimethylsulfoxide (DMSO) at stock concentrations of 100 mM, 10 mM and 5 mM, respectively, carefully sonicated, aliquoted and stored at −20 °C; appropriate dilutions of these solutions were made in saline (NaCl 0.9%) before injection. The amount of DMSO present in the solutions prepared for i.p. injections never exceeded 0.6 μl per mouse, and in all cases, control animals were injected with equivalent amounts of vehicle. KW-6002 (3 mg/kg/day, 0,025% methylcellulose) was diluted in the drinking water. The concentration was adjusted so that the drug intake was maintained at 3 mg/kg of body weight per day.

2.3. Intraperitoneal injection procedures

Acute drug administration was performed through a single intraperitoneal (i.p) injection, control animals being injected with the equivalent amount of vehicle. WIN 55,212-2 was used at a dose of 1 mg/kg of body weight, which is known to activate CB_1R in the central nervous system after i.p injection (Yim et al., 2008); AM251 was used at 3 mg/kg, a dose known as appropriate to antagonize CB_1R in the brain after i.p. administration (Chambers et al., 2004; McLaughlin et al., 2005; Xi et al., 2006); SCH 58261 was injected at a dose of 1 mg/kg, which keepts selectivity for $A_{2A}R$ over A_1R (Monopoli et al., 1998) and was used before to quickly target brain located $A_{2A}Rs$ after i.p. injection (De Sarro et al., 1996; El Yacoubi et al., 2001; Fontinha et al., 2009). All i.p. injections were in a volume of 2 ml/kg of body weight.

2.4. Chronic treatment with KW-6002

KW-6002 dose was selected according to previous testing of the efficacy and selectivity of this drug after oral administration (Yang et al., 2007); it was diluted in the drinking water (3 mg/kg/day) and administered for 30 consecutive days, as it was done before in our