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Cannabinoid-induced changes in respiration of brain mitochondria

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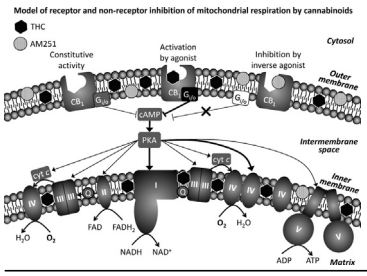
HIGHLIGHTS

- Cannabinoids are full or partial inhibitors of mitochondrial respiration.
- There is protective effect of antagonist/inverse agonist of cannabinoid receptor 1.
- Both receptor- and non-receptor action of cannabinoids influences respiration.

GRAPHICAL ABSTRACT



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ABSTRACT

Cannabinoids exert various biological effects that are either receptor-mediated or independent of receptor signaling. Mitochondrial effects of cannabinoids were interpreted either as non-receptor-mediated alteration of mitochondrial membranes, or as indirect consequences of activation of plasma membrane type 1 cannabinoid receptors (CB_1). Recently, CB_1 receptors were confirmed to be localized to the membranes of neuronal mitochondria, where their activation directly regulates respiration and energy production. Here, we performed in-depth analysis of cannabinoid-induced changes of mitochondrial respiration using both an antagonist/inverse agonist of CB_1 receptors, AM251 and the cannabinoid receptor agonists, Δ^9-tetrahydrocannabinol (THC), cannabidiol, anandamide, and WIN 55,212-2. Relationships were determined between cannabinoid concentration and respiratory rate driven by substrates of complex I, II or IV in pig brain mitochondria. Either full or partial inhibition of respiratory rate was found for the tested drugs, with an IC_{50} in the micromolar range, which verified the significant role of non-receptor-mediated mechanism in inhibiting mitochondrial respiration. Effect of stepwise application of THC and AM251 evidenced protective role of AM251 and corroborated the participation of CB_1 receptor activation in the inhibition of mitochondrial respiration. We proposed a model, which includes both receptor- and non-receptor-mediated mechanisms of cannabinoid action on mitochondrial respiration. This model explains both the inhibitory effect of cannabinoids and the protective effect of the CB_1 receptor inverse agonist.

Abbreviations: ADP, adenosine diphosphate; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1H-pyrazole-3-carboxamide; cAMP, cyclic adenosine monophosphate; CB, cannabinoid receptor; DMSO, dimethyl sulfoxide; FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; IC_{50}, drug concentration that is required for 50% inhibition; K_i, affinity for receptor; MiR05, mitochondrial respiration medium; OXPHOS, oxidative phosphorylation; PKA, protein kinase A; THC, Δ^9-tetrahydrocannabinol; TMPD, tetramethyl-p-phenylenediamine; WIN 55,212-2, R-(+)-WIN 55,212, R-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone.

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1. Introduction

Besides their crucial role in the generation of adenosine-5′-triphosphate (ATP), mitochondria are involved in other important processes, such as regulation of free radicals, neurotransmitters, calcium, and apoptosis (Mattson et al., 2008). Thus, mitochondria represents a possible drug target with unexplored therapeutic and toxicological potential (Kanabus et al., 2014; Szeto, 2014). Cannabinoids, a class of chemical compounds that are specifically recognized by the cannabinoid receptors, are known to inhibit mitochondrial respiration (Bénard et al., 2012; Whyte et al., 2010). The physiological functions of the endocannabinoid system are complex and involve, among other functions, motor coordination, memory, appetite, pain modulation, neuroprotection, cognitive functions, affective states, and maintenance of energy homeostasis (Fišar, 2012; Marsicano and Lutz, 2006; Morena and Campolongo, 2014; Piscitelli and Di Marzo, 2012; Silvestri and Di Marzo, 2013; Trezza and Campolongo, 2013). Hence, research on the therapeutic potential of drugs modulating the endocannabinoid system is extensive (Aso and Ferrer, 2014; Maione et al., 2013; Pacher et al., 2006; Pacher and Kunos, 2013; Pertwee, 2009).

1.1. Cannabinoids

Cannabinoids can be categorized into (i) phytocannabinoids, e.g., Δ^9-tetrahydrocannabinol (THC) and cannabidiol; (ii) endocannabinoids, e.g., anandamide and 2-arachidonoylglycerol; and (iii) synthetic cannabinoids, e.g., WIN 55,212-2 and HU-210. Synthetic cannabinoids include potent cannabinoid receptor agonists and antagonists/inverse agonists. Antagonists were suggested to have the ability for minimization or prevention of side effects of phytocannabinoids. Rimonabant (SR141716A) and its analogue AM251 (Gatley et al., 1997) are examples of antagonist/inverse agonists of the type 1 cannabinoid receptor (CB_1).

The discovery of cannabinoid receptors (Devane et al., 1988) and the endocannabinoid system were the key events in recognizing cannabinoid receptors as the principal molecular target of cannabinoids (Pertwee et al., 2010). There are two types of G protein-coupled cannabinoid receptors, CB_1 and CB_2. CB_1 receptors are abundant in neurons, where they modulate neurotransmission. CB_2 receptors are expressed in cells of the immune system and mediate immunosuppressive effects; however, the functional presence of CB_2 receptors in central nervous system was demonstrated both in glia and at synapses (Morgan et al., 2009; Onaivi et al., 2006). Both receptor types are coupled to $G_{i/o}$ proteins, negatively to adenylyl cyclase and positively to mitogen-activated protein kinase. Furthermore, CB_1 receptors are connected *via* $G_{i/o}$ proteins to ion channels, negatively to N-type and P/Q-type calcium channels and positively to various types of potassium channels (Turu and Hunyady, 2010). The biochemical targets of cannabinoids include both CB_1 and CB_2 receptors along with downstream targets, which are regulated by chronic drug administration and receptor-independent effects.

Cannabinoids are lipophilic molecules that are incorporated and accumulated into the cell membrane lipid bilayer. The physicochemical properties of cannabinoids initially led to the assumption that they exert their effect *via* disturbance of the ordering of the lipid region of cell membranes (Leuschner et al., 1984; Mavromoustakos et al., 2001). Recently, it was confirmed that cannabinoids may influence lipid membrane domain formation (Tian et al., 2011) and activity of many membrane proteins in a receptor-independent mechanism (Maccarrone et al., 2011), including mitochondrial functions (Lenaz, 1987; Waczulikova et al., 2007 Zhang et al., 2013). The exact mechanisms of the action of cannabinoids have not been explained and the potential for modulators of the endocannabinoid system to induce both beneficial and harmful effects should be studied (Fowler et al., 2010; Sarne et al., 2011). In the brain, CB_1 receptor mediates the effects of endocannabinoids on neuronal transmission, plasticity and functions (Kano et al., 2009; Katona et al., 1999; Piomelli, 2003; Szabo et al., 1998). Functionally relevant levels of CB_1 receptors are also present in astrocytes (Stella, 2010) and participate in the release of inflammatory mediators, control of cellular metabolism, modulation of neuron–astrocytes communication and glutamate signaling.

1.2. Mitochondrial respiratory chain

Process of oxidative phosphorylation (OXPHOS) is performed by means of electron flow between complex I, III and IV or II, III and IV. Complex I, III and IV pump protons through inner mitochondrial membrane and proton gradient is utilized for ATP synthesis by ATP synthase (complex V). Respiratory complexes are multisubunit enzymes. There is electron transfer between the complexes mediated by lipid-soluble ubiquinone (between complex I or II and complex III) and water soluble cytochrome c (between complex III and complex IV). Complex III is known to exist in the membrane as a dimer. The OXPHOS protein complexes I, II, III and IV associate into stable respiratory supercomplexes (Chaban et al., 2014). In mammalian mitochondria, almost all the complex I are assembled into supercomplexes comprising complexes I and III_2 and up to four copies of complex IV (Schägger and Pfeiffer, 2000), these are called respirasomes. Recent studies have reported that dynamic supercomplex assembly determines optimal electron flux from different substrates through the respiratory chain (Acin-Perez and Enriquez, 2014; Chaban et al., 2014; Lapuente-Brun et al., 2013). It is proposed that the lipids (cardiolipin mainly) are required for the stability of the supercomplexes and also serve as a diffusion microdomain for the ubiquinone (Paradies et al., 2014; Pfeiffer et al., 2003).

Both, individual complexes and respirasome activities seem to be modulated by mitochondrial membrane potential and phosphorylation state of protein subunits (Genova and Lenaz, 2014). Cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA), present in subcellular compartments, including mitochondria (Sardanelli et al., 2006), phosphorylates mitochondrial proteins and plays a central regulatory role in energy-supplying and energy-requiring processes (Rosca and Hoppel, 2010). Complex I (Papa et al., 2012; Technikova-Dobrova et al., 2001; Yadava et al., 2008) and complex IV (Bender and Kadenbach, 2000) are the main targets for the regulation of oxidative phosphorylation by PKA.

1.3. Cannabinoids and mitochondria

Early studies suggest that cannabinoids affect mitochondrial function *via* lipophilic action (Bartova and Birmingham, 1976; Martin, 1986). Recently, endocannabinoid receptor signaling has been demonstrated to modulate numerous concomitant pathological processes, including neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress (Aso and Ferrer, 2014). Endocannabinoids, such as anandamide and 2-arachidonoylglycerol, were found to alter mitochondria-dependent signal transduction and thus participate in the regulation of energy homeostasis and apoptosis (Lipina et al., 2014). The upregulation of endocannabinoids may precede mitochondrial dysfunction in neurodegenerative disorders (Alger and Tang, 2012; Fagan and Campbell, 2014). Some effects of endocannabinoids are