The endocannabinoid 2-arachidonoylglicerol decreases calcium induced cytochrome c release from liver mitochondria

Patrizia Zaccagnino · Susanna D’Oria · Luigi Luciano Romano · Almerinda Di Venere · Anna Maria Sardanelli · Michele Lorusso

Received: 6 February 2012 / Accepted: 7 March 2012 / Published online: 22 March 2012 © Springer Science+Business Media, LLC 2012

Abstract 2-Arachidonoylglicerol (2-AG) is an endocannabinoid that mimics the pharmacological effects of Δ⁹-tetrahydrocannabinol, the psychoactive component of the plant Cannabis sativa. It is present in many mammalian tissues, such as brain, liver, spleen, heart and kidney, where it exerts different biological effects either receptor mediated or independently of receptor activation. This work analyzes the effects of 2-AG on liver mitochondrial functions. It is shown that 2-AG causes a relevant decrease of calcium induced cyclosporine A sensitive cytochrome c release from mitochondria, a process representing an early event of the apoptotic program. Cyclosporin sensitive matrix swelling and ROS production measured under the same conditions are, on the contrary, almost unaffected or even enhanced, respectively, by 2-AG. Furthermore, 2-AG is found to stimulate resting state succinate oxidase activity and to inhibit oligomycin sensitive F₃,F₇ ATP synthase activity. All these effects are apparently associated with 2-AG dependent alteration in the fluidity of the mitochondrial membranes, which was measured as generalized polarization of laurdan fluorescence.

Keywords Endocannabinoids · 2-Arachidonoylglicerol · Mitochondrial calcium overload · Mitochondrial membrane fluidity · Cytochrome c release · F₃,F₇ ATP synthase

Introduction

The endocannabinoid system is an ubiquitous signalling network composed of specific receptors and their endogenous agonistic ligands, the so-called endocannabinoids. Endocannabinoids are bioactive lipid molecules, that comprise amides, esters and ethers of long chain polyunsaturated fatty acids and mimic the pharmacological actions of the exogenous cannabinoid Δ⁹-tetrahydrocannabinol, the primary psychoactive ingredient of hashish and marijuana (Gaoni and Mechoulam 1971).

Endocannabinoids are involved in a different set of physiological and pathological processes such as immunomodulation, analgesia, cancer, appetite, epilepsy (Di Marzo and Petrocellis 2006; Zias et al. 1993; Di Marzo 1998; Piomelli 2005; Pacher et al. 2006) and produce their effects by binding to distinct G protein-coupled receptors identified as the cannabinoid CB1 and CB2 receptors (Devane et al. 1988; Matsuda et al. 1990; Munro et al. 1993). It has been recently reported that the actions of endocannabinoids may also be mediated by two orphan G protein-coupled non-CB1/CB2 receptors, GPR55 and GPR119 (Ryberg et al. 2007; Brown 2007). Endocannabinoids are produced on demand through cleavage of membrane phospholipids precursors. They are active near the site of their synthesis and, after exerting their action, are hydrolyzed by specific enzymes.

The two most studied members of the endocannabinoid family are N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG).
2-AG is a monoacylglycerol with an esterified arachidonic acid at the sn-2 position of glycerol backbone. The enzymatic cleavage of phosphatidylinositol by phospholipase C appears the major source of the 2-AG precursor diacylglycerol, that subsequently is hydrolyzed by a diacylglycerol lipase to produce 2-AG (Wang and Ueda 2009; Basavarajappa 2007; Bisogno et al. 2003; Di Marzo et al. 1999). 2-AG production is strongly stimulated by calcium (Kondo et al. 1998) or after membrane depolarization causing in turn an increase of intracellular Ca\(^{2+}\) levels (Kano et al. 2009).

2-AG first found in the central and peripheral nervous system (Sugiura et al. 1995; Stella et al. 1997), was subsequently demonstrated to be present in many other mammalian tissues such as liver, spleen, lung, heart, small intestine, kidney and colon (Kondo et al. 1998; Schmid et al. 2000; Pinto et al. 2002; Izzo et al. 2001). This broad distribution suggests it may play multiple roles with a number of target sites. Among the various receptor mediated biological responses produced, inhibition of adenyl cyclase activity (Stella et al. 1997; Franklin et al. 2003; Mukherjee et al. 2004), modulation of lymphocyte proliferation (Sipe et al. 2005), inhibition of the hepatic fibroblast (Julien et al. 2005) and cancer growth (Melck et al. 2000; Ligresti et al. 2003) have been reported.

Recent reports have, however, shown that endocannabinoids exert definite biological effects within the cell, that are independent of cannabinoid receptor activation. In particular, mitochondria appear to be involved in the intracellular anandamide and 2-AG action (Zaccagnino et al. 2011; Catanzaro et al. 2009; Siegmund et al. 2007).

These observations prompted us to carry out the present study aimed to better understand the effect of 2-AG on liver mitochondrial functions. We report here that 2-AG causes a substantial reduction of Ca\(^{2+}\) induced cytochrome c release from mitochondria, thus apparently conferring resistance of mitochondria to proapoptotic signals. This effect, together with those exerted on oxidative phosphorylation enzymes, is apparently associated with 2-AG induced alterations of lipid membrane ordering.

Materials and methods

Chemicals

Alamethicin, safranin-O, 6-Dodecanoyl-2-dimethylaminonaphthalene (Laurdan), p1,p2-Di (adenosine-5') pentaphosphate pentalithium (Ap5) salt were purchased from Sigma (St. Louis, MO, USA). Cyclosporin A (CsA) was purchased from Calbiochem (San Diego, CA, USA). Hexokinase, phosphoenolpyruvate (PEP), L-lactate dehydrogenase (LDH), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G6P-DH) and horseradish peroxidase (HRP) were from Roche Diagnostics Corporation (Indianapolis, IN, USA). 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) and calcium green-5N were purchased from Invitrogen–Molecular Probes (Eugene, OR, USA). 2-arachidonoylglycerol (2-AG) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Mouse anti-cytochrome c antibody was purchased from Zymed Laboratories Invitrogen Immunodetection (Carlsbad, CA, USA).

Isolation of rat liver mitochondria

Animal maintenance, handling and sacrifice were conducted as recommended by the institutional laboratory animal committee of the University of Bari.

Rat liver mitochondria were isolated by differential centrifugation using an isolation buffer containing 0.22 M mannitol, 75 mM sucrose, 10 mM HEPES (pH 7.4), 1 mM EDTA and 0.25 mM PMSF as reported by Ito et al. (Ito et al. 1985). The final mitochondrial pellet was resuspended in the same isolation buffer at a protein concentration of 50–60 mg/ml as determined by the Biuret method, using bovine serum albumin as standard.

Detection of cytochrome c release

Cytochrome c release from freshly isolated mitochondria (0.25 mg/ml) was measured as previously described (Zaccagnino et al. 2009). Mitochondria were suspended in a medium containing 75 mM sucrose, 50 mM KCl, 30 mM TRIS-Cl, (pH 7.4), 2 mM KH2PO4, 5 mM MgCl2 and 20 μM EGTA (Buffer A), supplemented with 10 mM succinate (plus 2 μg/ml rotenone) and incubated for 2 min at 25 °C. Where indicated 20 μM 2-AG, 180 μM Ca\(^{2+}\) and 2 μM CsA were added. Densitometric analysis of the bands were carried out by using Quantity One-4.4.1 imaging software (Bio-Rad Laboratories).

Mitochondrial swelling

Mitochondria were suspended at 0.25 mg/ml in a medium containing 75 mM sucrose, 50 mM KCl, 30 mM TRIS-Cl, (pH 7.4), 2 mM KH2PO4, 10 mM succinate and 2 μg/ml rotenone. Swelling was triggered by the addition of 180 μM Ca\(^{2+}\) and changes in absorbance of the mitochondrial suspension were monitored at 540 nm in an Agilent 8453 diode-array spectrophotometer.

Detection of mitochondrial H2O2 production

The rate of mitochondrial H2O2 production was estimated by measuring the fluorescence increase (excitation at 475 nm, emission at 525 nm) induced by H2O2 oxidation.