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Inhibition of leukocyte function and interleukin-2 gene expression by 2-methylarachidonyl-(2′-fluoroethyl)amide, a stable congener of the endogenous cannabinoid receptor ligand anandamide

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Abstract

Arachidonylethanolamide (anandamide, AEA) has been identified as an endogenous ligand for cannabinoid receptors CB1 and CB2. Characterization of the direct cannabimimetic actions of anandamide has been hampered by its short duration of action and rapid degradation in in vivo and in vitro systems to arachidonic acid, a precursor in the biosynthesis of a broad range of biologically active molecules. In the present studies, we utilized 2-methylarachidonyl-(2′-fluoroethyl)amide (F-Me-AEA), an analog of anandamide resistant to enzymatic degradation, to determine whether F-Me-AEA modulated T cell function similar to that of plant-derived cannabinoids. Indeed, F-Me-AEA at low micromolar concentrations exhibited a marked inhibition of phorbol ester plus calcium ionophore (PMA/Io)-induced IL-2 protein secretion and steady state mRNA expression. Likewise, a modest suppression of the mixed lymphocyte response was observed in the presence of F-Me-AEA indicating an alteration in T cell responsiveness to allogeneic MHC class II antigens. F-Me-AEA was also found to modestly inhibit forskolin-stimulated adenylate cyclase activity in thymocytes and splenocytes, a hallmark of cannabinoid receptor agonists. Further characterization of the influence of F-Me-AEA on the cAMP signaling cascade revealed an inhibition of CREB-1/ATF-1 phosphorylation and subsequently, an inhibition of CRE DNA binding activity. Characterization of nuclear binding proteins further revealed that NF-AT and, to a lesser extent, NF-κB DNA binding activities were also suppressed. These studies demonstrate that F-Me-AEA modulates T cell function in a similar manner to plant-derived and endogenous cannabinoids and therefore can be utilized as an amidase- and hydrolysis-resistant endogenous cannabinoid.

Keywords: Anandamide; cAMP; Cannabinoid; Interleukin-2; T cells

Abbreviations: F-Me-AEA, 2-methylarachidonyl-(2′-fluoroethyl)amide; 2-Ara-Gl, 2-arachidonyl-glycerol; CB1, cannabinoid receptor type 1 (brain receptor); CB2, cannabinoid receptor type 2 (peripheral receptor); IL-2, interleukin-2; AA, arachidonic acid; Δ^9-THC, delta-9-tetrahydrocannabinol; CBN, cannabinol; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor for immunoglobulin κ chain in B cells; AP-1, activator protein 1; CREB, cAMP response element binding protein; CRE, cAMP response element; cAMP, cyclic adenosine 3′:5′-monophosphate; PKA, protein kinase A; PMA, phorbol-12-myristate-13-acetate; Io, ionomycin; PMSF, phenyl-methylsulfonyl fluoride; BCS, bovine calf serum; IS mRNA, internal standard mRNA; DEAE, diethyl aminoethyl; TBS, Tris-buffered saline; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcriptase-polymerase chain reaction; EMSA, electrophoretic mobility shift assay; CsA, cyclosporin A; FSK, forskolin.

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Introduction

The isolation and cloning of first the brain cannabinoid receptor (Matsuda et al., 1990), CB1, and subsequently the peripheral cannabinoid receptor (Munro et al., 1993), CB2, initiated a search for endogenous cannabinoid receptor ligands. By competitive binding, arachidonylethanolamide, termed anandamide (AEA), was the first putative endogenous cannabinoid receptor ligand to be identified (Devane et al., 1992). AEA is a natural eicosanoid originally isolated from porcine brain homogenates, and later identified in rat spleen, human spleen, human dendritic cells, and mouse peritoneal macrophages (Di Marzo et al., 1996; Felder et al., 1996; Matias et al., 2002; Schmid et al., 1997), which exhibits binding affinity for both CB1 and CB2.

Measurements of CB1 and CB2 steady state mRNA expression demonstrate that CB2 is the predominant receptor form expressed by leukocytes; however, it is notable that CB1 is also expressed in some primary leukocyte preparations such as mouse splenocytes and human peripheral blood leukocytes (Bouaboula et al., 1993; Kaminski et al., 1992; Schatz et al., 1997). To date, the greatest cell-type sensitivity to cannabinoids has been observed on macrophage and T cell function (Burnette-Curley and Cabral, 1995; Cabral and Vasquez, 1992; Cabral et al., 1991; Condie et al., 1996; Puffenbarger et al., 2000; Schatz et al., 1993, 1997). Conversely, B cells appear to be rather refractory to the inhibitory effects associated with high doses of cannabinoids (Schatz et al., 1993) while exhibiting modest stimulatory effects to low concentrations of cannabinoids (Derocq et al., 1995).

A number of studies aimed at characterizing the immunomodulatory activity of AEA have demonstrated that this fatty acid ethanolamide suppressed LPS-induced nitric oxide production by mouse peritoneal macrophages (Coffey et al., 1996), macrophage-mediated killing of tumor necrosis-sensitive L929 fibroblasts (Cabral et al., 1995), and LPS-induced cytokine mRNA expression in rat microglial cells (Puffenbarger et al., 2000). In addition, AEA has been shown to decrease mitogen-induced T and B cell proliferation (Schwarz et al., 1994) and suppress T lymphocyte migration (Joseph et al., 2004). All of the aforementioned effects are typically produced by classical cannabinoids and therefore the results are consistent with the interpretation that AEA is an endogenous cannabinoid receptor ligand. In spite of these findings, it has been difficult to determine whether AEA exerts its effects directly or via a metabolite(s). AEA and other fatty acid ethanolamides are highly labile molecules that are susceptible to enzymatic degradation by amidases associated with cell membranes (Deutsch and Chin, 1993) and binding affinity to cannabinoid receptors is increased in the presence of inhibitors of amidase activity such as PMSF (Adams et al., 1995). The inactivation of AEA primarily occurs by a re-uptake mechanism via anandamide membrane transporter (AMT), after which AEA is hydrolyzed into arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996; Di Marzo et al., 1994; Giang and Cravatt, 1997). AEA is also a substrate for cyclooxygenase-2 (COX-2), which converts AEA into prostaglandin E2 ethanolamide (Yu et al., 1997). Based on the fact that AEA is highly labile, the objective of the present studies was to evaluate the immunomodulatory activity of a synthetic AEA congener, F-Me-AEA. F-Me-AEA is resistant to hydrolysis and enzymatic degradation by amidases and its CB1 binding activity is not significantly different in the presence and absence of PMSF (Adams et al., 1995). The studies described here provide a detailed characterization of the effects of F-Me-AEA on various T cell responses and provide insight into the mechanism of action of AEA.

Materials and methods

Animals. Virus-free female B6C3F1 mice, 6 weeks of age, were purchased from Charles River (Dortage, MI). On arrival, mice were randomized, transferred to plastic cages containing sawdust bedding (5 mice per cage), and quarantined for 1 week. Mice were given food (Purina Certified Laboratory Chow) and water ad libitum and were not used for experimentation until their body weight was 17–20 g. Animal holding rooms were kept at 21–24 °C and 40–60% relative humidity with a 12 h light/dark cycle.

Reagents and cell culture. All reagents were purchased from Sigma (St. Louis, MO) unless otherwise noted. 2-Methyl-arachidonyl-(2′-fluoroethyl)amide (Me-F-AEA) was synthesized by Dr. R. Razdan. F-Me-AEA was solubilized in absolute ethanol, aliquoted, and stored under nitrogen at −80 °C. Working solutions were prepared freshly just prior to addition to culture.

Spleens were isolated aseptically and made into single cell suspensions as described previously (Kaminski et al., 1994). The splenocytes and thymocytes were cultured in RPMI 1640 medium (Gibco BRL/Invitrogen, Carlsbad, CA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol and 5% bovine calf serum (BCS, Hyclone, Logan, UT). Leukocytes (1×10^6 cells/ml) were pretreated with F-Me-AEA (1, 5, 10 and 20 μM), cyclosporin A (CsA, 1 μM), vehicle (VH, 0.1% ethanol), or media alone (NA) for 15 min and then stimulated with phorbol 12-myristate 13-acetate (PMA, 40 nM) plus ionomycin (Io, 0.5 μM). In all cases, leukocytes were cultured at 37 °C in 5% CO_2.

Quantitative competitive reverse transcriptase-polymerase chain reaction (RT-PCR). All reagents used for RT-PCR were of molecular biology grade and were purchased from Promega Co. (Madison, WI) unless otherwise noted. Total RNA was isolated using Tri Reagent (Molecular Research Center, Cincinnati, OH). IL-2 steady state mRNA expression was quantified by quantitative competitive RT-PCR as described previously (Condie et al.,