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A COX-2 metabolite of the endogenous cannabinoid, 2-arachidonyl glycerol, mediates suppression of IL-2 secretion in activated Jurkat T cells

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ARTICLE INFO

Article history:
Received 28 January 2008
Accepted 5 May 2008

Keywords:
2-Arachidonyl glycerol
2-AG
2-AG ether
IL-2
T cell
Cannabinoid
Endocannabinoid
Jurkat
COX
Cyclooxygenase
Anandamide

ABSTRACT

Previous studies from this laboratory have demonstrated that a COX-2 metabolite of the endogenous cannabinoid, 2-arachidonyl glycerol (2-AG), inhibits IL-2 secretion in activated T cells through PPARγ activation independent of the cannabinoid receptors, CB1/CB2. Because numerous cyclooxygenase (COX) products have been shown to activate PPARγ, the primary purpose of the present studies was to determine the role of COX metabolism in the inhibition of IL-2 secretion by 2-AG. Pretreatment with nonselective and COX-2-specific inhibitors completely abrogated 2-AG-mediated suppression of IL-2 secretion. In contrast, pretreatment with COX-1-specific inhibitors had no effect upon 2-AG-mediated inhibition of IL-2 secretion. Interestingly, the current studies also demonstrate that while the potency of 2-AG is comparable between human Jurkat T cells and murine splenocytes, anandamide (AEA) is markedly more potent in suppressing IL-2 production in Jurkat T cells compared to murine splenocytes. Additionally, the present studies also demonstrate that COX-2 protein is readily detectable in resting Jurkat T cells, which is in contrast to resting murine splenocytes in which COX-2 protein is virtually undetectable. Furthermore, COX-2 protein and mRNA levels are significantly increased over basal levels by 2 h following activation of Jurkat cells, whereas increases in COX-2 protein in murine splenocytes are not observed until 4 h after cellular activation. These studies suggest that the potency of AEA in the suppression of IL-2 secretion may correlate with COX-2 protein levels in different T cell models. The present studies are also significant in that they demonstrate 2-AG-mediated inhibition of IL-2 secretion is dependent upon COX-2 metabolism.

1. Introduction

2-Arachidonyl glycerol (2-AG) is an endogenous arachidonic acid derivative and a high-affinity agonist of the cannabinoid receptors, CB1 and CB2, and is hence termed an endocannabinoid [1]. 2-AG has been implicated as an endogenous modulator of the immune system due to its detection in a variety of different cellular elements of the immune system, including dendritic cells, macrophages, microglia, and lymphocytes [2–5]. Moreover, activation of macrophages and other

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0006-2952/$ – see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.bcp.2008.05.005

immune cell types causes a rapid and robust increase in 2-AG levels, suggesting that 2-AG may play a role in immune regulation [4,6–8]. Further evidence for an immunoregulatory role of 2-AG comes from a number of published studies reporting effects by 2-AG in leukocytes and on immunological responses, including calcium influx in HL-60 cells, enhancement of antibody formation in murine splenocytes, and induction of the migration of human peripheral blood monocytes and HL-60 cells [9–11]. In addition, 2-AG also inhibits cytokine release by leukocytes as demonstrated in murine splenocytes, rat microglial cells, and J774 macrophages [12–15].

2-AG has been shown to be hydrolyzed into arachidonic acid and glycerol by two enzymes: monoacylglycerol (MAG) lipase and fatty acid amide hydrolase (FAAH) [16,17]. FAAH is also thought to be the main enzyme responsible for the hydrolysis of anandamide (AEA), another structurally-related ligand of the cannabinoid receptors [18]. In addition to its hydrolysis by MAG lipase and FAAH, 2-AG has also been shown to be metabolized by cyclooxygenase (COX) 2 [19]. COX has two subtypes, 1 and 2, which are similar structurally, but differ in regulation of expression, tissue distribution, and to a certain extent, their substrates [20,21]. While COX-1 is constitutively expressed in most cell types, COX-2 expression is generally restricted to activated leukocytes. Metabolism of arachidonic acid by COX-1 and COX-2 results in the production of a variety of eicosanoids, including prostaglandins and thromboxanes. Like arachidonic acid, 2-AG is also a substrate for COX-2 and PGE synthase and can be metabolized into prostaglandin E2-glyceryl ester, as well as other COX products [22].

Because a number of COX products including cyclopentenone prostaglandins have been found to activate PPARγ and our recent studies have demonstrated that 2-AG treatment results in the suppression of IL-2 secretion through PPARγ activation, the objective of the present studies was to determine the role of COX metabolism in the suppression of IL-2 secretion by 2-AG [23,24]. IL-2 is an autocrine/paracrine factor secreted by activated T cells and is important for T cell survival, proliferation, and in some cases, differentiation. As such, IL-2 is critically involved in the development of an adaptive immune response. We have demonstrated here that impairment of IL-2 secretion by 2-AG is dependent upon COX-2 metabolism. The current studies are the first to demonstrate that inhibition of IL-2 secretion upon 2-AG treatment is mediated by a COX-2 metabolite of 2-AG rather than the parent molecule itself. Moreover, the demonstration that eicosanoids derived from 2-AG modulate lymphocyte function signifies the biological relevance of this new class of COX-2 metabolites.

2. Materials and methods

2.1. Materials

2-Arachidonyl glycerol, SR141716A and SR144528 were provided by the National Institute on Drug Abuse. 2-Arachidonyl glycerol ether, FR122047, SC560, and NS398 were purchased from Cayman Chemical (Ann Arbor, MI). Piroxicam was purchased from Tocris Cookson (Ellisville, MO). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Animals and cell culture

Female B6C3F1 mice, 6 weeks of age, were purchased from Charles River Laboratories (Wilmington, MA). Studies requiring animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University. Spleens were isolated aseptically and processed into single-cell suspensions (1×10^6 cells/ml). Cells were cultured in RPMI 1640 supplemented with 100 units penicillin/ml, 100 units streptomycin/ml, 50 μM 2-mercapoethanol (2-ME), and 2% bovine calf serum (BCS). Jurkat E6-1 T cells were purchased from the American Type Culture Collection (Manassas, VA). Jurkat cells were cultured in RPMI 1640 supplemented with 100 units streptomycin/ml, 100 units penicillin/ml, 10 mM nonessential amino acids, 100 mM sodium pyruvate, and 10% BCS.

2.3. IL-2 ELISA

Jurkat cells (5×10^5 cells/ml) or splenocytes (1×10^6 cells/ml) were cultured in triplicate in 48-well culture plates (800 μl/well) in complete RPMI containing 2% BCS. The cells were either pretreated for 30 min with an inhibitor, or left untreated prior to treatment with 2-AG. Following a 30 min incubation, the cells were then stimulated with 40 nM PMA and 0.5 μM ionomycin (PMA/Io). The supernatants were collected 24 h after stimulation and IL-2 protein was quantified using the sandwich ELISA method as previously described [25]. The IL-2 standard (mouse or human recombinant IL-2), purified rat anti-mouse or mouse anti-human IL-2 antibody, and biotinylated anti-mouse or anti-human IL-2 antibody were purchased from BD Pharmingen (San Diego, CA).

2.4. Protein isolation

Jurkat cells (2.5×10^7 cells) or splenocytes (2×10^7 cells) were treated with PMA/Io for 2, 4, 8 or 12 h. At the end of the culture period, the cells were centrifuged and suspended in 100 μl of RIPA buffer (phosphate buffered saline containing 1% Igepal, 0.5% sodium deoxycholate and 0.1% SDS) for 5 min at ambient temperature. The suspension was then sonicated and incubated on ice for 30 min. The cells were then centrifuged at $10{,}000 \times g$ for 20 min at 4 °C and the supernatant was retained. The protein concentration was quantified by BCA assay (Sigma, St. Louis, MO).

2.5. Gel electrophoresis and western analysis

Protein (50 μg Jurkat protein; 30 μg splenocyte protein) was diluted with loading buffer (0.0625 M Tris, 2% SDS 10% glycerol, 0.01% bromophenol blue, and 1% 2-ME), loaded into an 8% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and incubated with blocking buffer (4% dry non-fat milk in 0.05% TBS–Tween 20 for Jurkat protein; also added 1%