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Review

Analysis of endocannabinoids, their congeners and COX-2 metabolites☆

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ABSTRACT

Since the discovery of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) in the early 1990s, the endocannabinoid system has been implicated in a wide array of physiological processes, such as control of food intake and energy balance, fertility and obesity. As the importance of this system becomes apparent, there is a tremendous need for robust, sensitive and efficient analytical methodology for the examination of the endocannabinoids, their congeners and putative metabolites. This review will summarize quantitative analytical methodology as reported in the literature from 1992 to present for the analysis of endocannabinoids and related compounds.

Contents

1. Introduction

Endocannabinoids are defined as endogenously produced compounds that bind to and functionally activate the cannabinoid receptors, CB1 and CB2 [1]. Since the discovery of anandamide (AEA) in 1992 [2], approximately seven additional endogenous compounds that activate either of the CB receptors have been identified from mammalian tissues [3–9]. Table 1 lists known endocannabinoids and their structures. Of these compounds, AEA and the monoacylglycerol, 2-arachidonoylglycerol (2-AG) have been the subject of the majority of scientific investigation.

Several enzymes regulate the generation and degradation of these compounds. The most well-characterized enzymes involved in endocannabinoid degradation are fatty acid amide hydrolase (FAAH), which cleaves AEA into free arachidonic acid and ethanolamine, and monoacylglycerol lipase (MAGL), which hydrolyzes monoacylglycerols (MAGs), including 2-AG, to gener-

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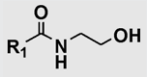
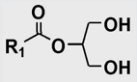
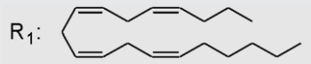
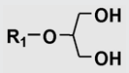
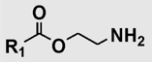
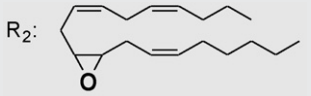
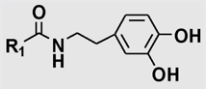
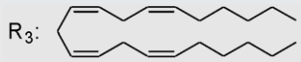
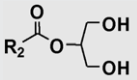
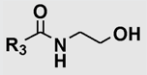
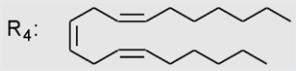
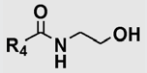
Table 1
Structure of current endocannabinoids.

Compound	Structure	R
Anandamide [2]		
2-Arachidonoyl glycerol [3,4]		
Noladin [5]		
Virodhamine [9]		
N-Arachidonoyl dopamine [6]		
2-Epoxyeicosatrienoyl glycerol [7]		
Docosatetraenoyl ethanolamide [8]		
Dihomo-γ-linolenoyl ethanolamide [8]		

Note: The epoxide oxygen in R_2 may be between carbons 11 and 12 (shown) or between carbons 14 and 15.

ate free arachidonic acid and glycerol. Diacylglycerol lipase type-α (DAGL-α) has been shown to generate 2-AG from diacylglycerols, particularly 1-stearoyl, 2-arachidonoylglycerol [10,11] while 2-AG and AEA have been shown to be substrates of oxygenating enzymes. In 1997, Yu et al. reported that AEA is a substrate for the cyclooxygenase-2 enzyme (COX-2) but not COX-1 [12]. The oxygenated products included PGH_2-ethanolamide, which was shown to be isomerized by various PG synthases, resulting in a series of prostaglandin ethanolamides (PG-EAs). Later, Kozak et al. reported that 2-AG was similarly oxygenated by COX-2 (and not COX-1) and via PG synthases, gave rise to a series of prostaglandin glycerol esters (PG-Gs) [13].

The endocannabinoids given in Table 1, the enzymes discussed above (as well as putative endocannabinoid transporters), and the two characterized CB receptors comprise the endocannabinoid system. In the last decade, a significant body of evidence has developed implicating this system in a wide array of physiological processes, such as control of food intake and energy balance [14], emesis [15], fertility [16] and obesity [17]. Additionally, Nirodi et al. demonstrated that PGE_2-G induces Ca^{2+} mobilization in RAW cells [18] and, recently, Hu et al. showed PGE_2-G to have hyperalgesic properties *in vivo* and succeeded in recovering PGE_2-G from the hindpaw of mice [19].

Endocannabinoids are often the only species within a larger class of lipid biomolecules to display activity at either CB receptor. The non-cannabimimetic members of these molecular classes, however, have been shown to effect the actions of true endocannabinoids. For example, Ben-Shabat et al. reported that in competitive binding assays in CHO cells transfected with the CB2 receptor, the K_i of 2-AG alone was 1640 nM. A mixture of 2-AG, 2-linoleoylglycerol (2-LG) and 2-palmitoylglycerol displayed a K_i of 273 nM, despite the lack of activity from 2-LG and 2-palmitoylglycerol below 20 μM [20]. The lowering of K_i values in the presence of inactive compounds – termed the "entourage effect" – was seen for 2-AG binding to CB1 as well.

Given the existence of several endocannabinoids throughout mammalian tissue and the realization that the endocannabinoid system is a highly relevant physiological pathway whose modulation may prove to be efficacious for the treatment of a wide variety of pathophysiological conditions, there is a tremendous need for robust, sensitive and efficient analytical methodology for the examination of the endocannabinoids, their congeners and putative metabolites. The whole of the signaling cascade must be taken into account if the fundamental nature of the endocannabinoid system is to be elucidated. It is the goal of this review to summarize quantitative analytical methodology as reported in the literature from 1992 to present for the analysis of endocannabinoids and related compounds.

2. Sample preparation and purification

Sample preparation for endocannabinoid analysis from tissue typically consists of homogenization of the tissue of interest in an organic solvent followed by further purification/isolation of the analytes via open-bed chromatography or solid phase extraction (SPE, also sometimes referred to as mini-columns). Purification of endocannabinoids from cell media is usually performed by SPE techniques. Despite the high degree of specificity afforded by current detection techniques, specifically liquid chromatography in-line with tandem mass spectrometry, it is desirable to subject samples to an efficient purification process prior to such analysis. This is especially so for samples from biological matrices, where the number of endogenous lipids and low molecular weight compounds, all potential interferants for chromatographic and mass spectrometric analysis, is significant.

Some researchers have employed an intricate purification strategy. For example, Kirkham et al. homogenized dissected mouse hypothalamus and limbic forebrain in a solution of chloroform–methanol–Tris HCl (50 mM), 2:1:1, v/v [21]. The homogenate is centrifuged and the organic layer removed. The