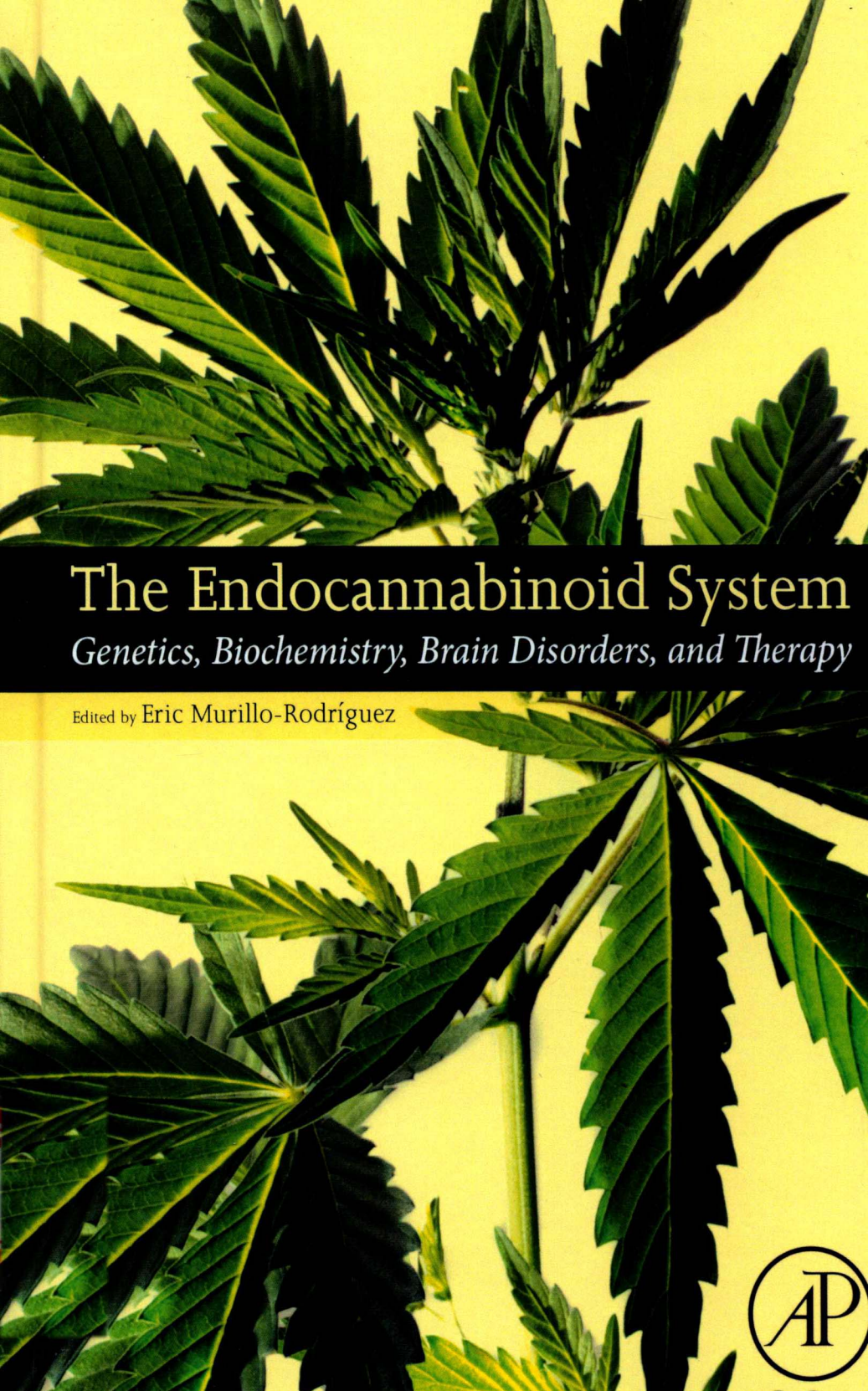

The Endocannabinoid System

Genetics, Biochemistry, Brain Disorders, and Therapy

Edited by Eric Murillo-Rodríguez

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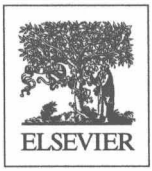
ERIC MURILLO-RODRÍGUEZ, PhD
Universidad Anáhuac Mayab, Mexico



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THE ENDOCANNABINOID SYSTEM

LIST OF CONTRIBUTORS

John C. Ashton
University of Otago, Dunedin, New Zealand

Balapal S. Basavarajappa
New York State Psychiatric Institute, New York, NY, United States; Columbia University, New York, NY, United States; Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, United States

Natalia Battista
University of Teramo, Teramo, Italy

Megan J. Dowie
University of Auckland, Auckland, New Zealand

Andrea Giuffrida
University of Texas Health Science Center, San Antonio, TX, United States

Michelle Glass
University of Auckland, Auckland, New Zealand

Bernard Le Foll
Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada

F. Markus Leweke
Heidelberg University, Mannheim, Germany

Mauro Maccarrone
Campus Bio-Medico University of Rome, Rome, Italy; European Center for Brain Research/IRCCS Santa Lucia Foundation, Rome, Italy

Alex Martinez
University of Texas Health Science Center, San Antonio, TX, United States

Cathrin Rohleder
Heidelberg University, Mannheim, Germany

Jose M. Trigo
Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada

PREFACE

In January 2017, the National Academy of Sciences, Engineering and Medicine (NASEM) of the United States published a new report on the health effects of cannabis (http://nationalacademies.org/hmd/reports/2017/health-effects-of-cannabis-and-cannabinoids.aspx). This 500-page document provides an overview of the research of the past 15 years on the health impacts of cannabis and cannabis-derived products, covering a vast amount of scientific material—more than 10,000 published articles—in a rigorous and balanced manner. The report discusses the health risks associated with cannabis use as well as its potential therapeutic effect, and draws conclusions on almost 100 highly relevant topics, ranging from lung cancer and diabetes to schizophrenia and addiction. The sheer size and span of this document bears testimony to the growing importance of its subject matter. For many years now, cannabis has been the most popular illicit drug in the United States and Europe. A recent nationwide survey estimates that 22.2 million Americans aged 12 years and older consumed cannabis in the past 30 days, in the vast majority of cases, for recreational purposes. Although still relatively limited, medical use will undoubtedly expand in the near future as acceptance and accessibility of cannabis as a medicine increase. Basic and clinical research has grown in parallel with public and medical interest, such that more than 24,000 scientific articles have been published on cannabis and the cannabinoids since publication of the previous NASEM report in 1999.

Far from writing the last word on the health impact of cannabinoids, the new NASEM review emphasizes the need to better understand how cannabis and its chemical constituents interact with the human body, and to consolidate new data gathered through animal and human research into solid scientific knowledge. The present book, edited by Dr. Eric Murillo-Rodríguez (at the University Anàhuac Mayab, Merida, Mexico), aims to address those very same needs. A distinguished group of authors, each an expert in their discipline, tackle a broad set of topics centered around the endogenous cannabinoid complex, the signaling system that mediates the action of Δ^9-tetrahydrocannabinol in cannabis.

The book starts with an overview of the biochemical mechanisms through which the endocannabinoid transmitters—anandamide and 2-arachidonoylglycerol (2-AG)—are produced and eliminated. Drs. Natalia Battista (University of Teramo, Italy) and Mauro Maccarrone (University of

Rome, Italy), outline the distinct enzyme pathways involved in the formation and degradation of these lipid-derived substances and highlight key knowledge gaps that remain to be filled to arrive at a full molecular understanding of endocannabinoid signaling. In the following chapter, Dr. Balapal Basavarajappa (New York State Psychiatric Institute and Columbia University, New York, USA) provides an introduction to CB_1 and CB_2 cannabinoid receptors, the G-protein-coupled receptors targeted by exogenous and endogenous cannabinoids. The chapter also describes the neuroanatomical localization of these receptors, along with the intracellular signaling cascades recruited by them.

After these two introductory chapters, the book develops into a series of small monographs, each centered on the roles played by endocannabinoid signals in human pathological conditions. Drs. Andrea Giuffrida and Alex Martinez (both at the University of Texas Health Science Center, San Antonio, USA) summarize current views about the contribution of anandamide and 2-AG to Parkinson's disease. This balanced résumé of current knowledge is particularly welcome at a time when anecdotal evidence spread through social media has fueled public interest (and speculation) on the possible therapeutic utility of cannabis in this neurodegenerative disorder (https://www.youtube.com/watch?v=zNT8Zo_sfwo).

In the next chapter, Drs. Markus Leweke and Cathrin Rohleder (both at the Central Institute on Mental Health, Heidelberg, Germany) review the important and complex issue of the link between cannabis use and schizophrenia. The NASEM report devotes a substantial amount of space to this issue, which remains hard to disentangle after 30 years of intense epidemiological research. The thoughtful perspective offered by Drs. Leweke and Rohleder is that the pathophysiology of schizophrenia may be underpinned by a deficiency in anandamide-mediated signaling at CB_1 receptors, which implies that agents that heighten the actions of anandamide might be protective in this disorder. In Chapter 5, Dr. Michelle Glass and her collaborators (at the Universities of Otago and Auckland, New Zealand) take a broader view of the pathological roles of the endocannabinoid system and discuss multiple possible implications of this signaling complex in cognitive and motor dysfunction as well as in mood disorders. Lastly, Dr. Bernard Le Foll (University of Toronto, Canada) closes this interesting aperçu of endocannabinoid physiopathology with a thought-provoking section on the role of endocannabinoid signals in drug addiction. Summarizing the available preclinical evidence, Dr. Le Foll concludes that CB_1 receptors serve an important modulatory function in drug-seeking behavior, and that elevating

anandamide levels in the brain (for example, by preventing its enzyme-mediated degradation) may attenuate drug seeking.

In sum, this new book offers an interesting bird's-eye view of the many possible roles played by the endocannabinoid signaling system in brain psychopathology. Read alongside the 2007 NASEM report, it will represent a useful teaching and learning tool for students and scientists who enter this exciting and important field of investigation.

Daniele Piomelli
Departments of Anatomy and Neurobiology
Pharmacology and Biological Chemistry
University of California, Irvine

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I would like to express my gratitude to my family, with special love to my sister Linda and beautiful niece Shaula.

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Last but not the least: I beg forgiveness of all those who have been with me over the course of the years and whose names I have failed to mention.

Eric Murillo-Rodríguez

INTRODUCTION

The endocannabinoid system includes endogenous cannabinoidlike compounds such as anandamide; metabotropic receptors named CB_1 and CB_2; enzymes like fatty acid amide hydrolase, which synthesizes anandamide; as well as the anandamide membrane transporter.

Significant advances have been achieved by studying the physiological properties of the endocannabinoid system. The spectrum of evidence of the role of the endocannabinoid system includes neurobiological areas from gene expression to putative modulation of health issues.

At present, observations confirm that the endocannabinoid system displays critical functions by controlling a wide spectrum of physiological conditions. This book highlights the current evidence of the synthesis and degradation of endocannabinoids, as well as the role of the endocannabinoid system in neurodegenerative illness such as Parkinson disease. Moreover, the relevant function of the endocannabinoid system in mental disorders, including schizophrenia, and its critical role in human brain functions such as memory, motor behavior, and mood pathologies is highlighted and remarks on the current experimental evidence regarding the link between the endocannabinoid system and addiction are provided.

A number of world-leading scientists who are experts in the endocannabinoid area agreed to contribute to the book by providing current evidence of the relevance of the endocannabinoid system in multiple neurobiological functions. Thus this book deals with various aspects of the endocannabinoid system, from phenomena to molecular processes. I am sincerely grateful to all the contributors for their scientific contribution.

Eric Murillo-Rodríguez, PhD
Laboratorio de Neurociencias Moleculares e Integrativas
Escuela de Medicina, División Ciencias de la Salud
Universidad Anáhuac Mayab
Mérida, Yucatán, Mexico

CONTENTS

CHAPTER 1

Basic Mechanisms of Synthesis and Hydrolysis of Major Endocannabinoids

Natalia Battista[1], Mauro Maccarrone[2,3]
[1]University of Teramo, Teramo, Italy; [2]Campus Bio-Medico University of Rome, Rome, Italy; [3]European Center for Brain Research/IRCCS Santa Lucia Foundation, Rome, Italy

ENDOCANNABINOIDS AND THEIR ANALOGS

The isolation of the psychoactive ingredient of cannabis (*Cannabis sativa*), Δ^9-tetrahydrocannabinol, back in 1964 led almost 30 years later to the discovery of the endogenous lipophilic molecules, collectively termed endocannabinoids (eCBs), able to activate the same two G-protein-coupled receptors, type 1 (CB_1) and type 2 (CB_2) cannabinoid receptors (Maccarrone et al., 2015).

On the basis of their main role in controlling biological processes both in human health and disease, *N*-arachidonoylethanolamine (anandamide) (AEA) and 2-arachidonoylglycerol (2-AG) are presently recognized as the two main members of this family (Fig. 1.1). Other important ω-6 (n-6) fatty acid compounds with cannabimimetic properties, such as *N*-arachidonoyldopamine (NADA), 2-arachidonoylglycerylether (noladin ether), and *O*-arachidonoylethanolamine (virodhamine), have also been listed among these bioactive lipids, although their pharmacology and biological relevance remain to be clarified (Fezza et al., 2014). In addition, in the past 5 years it has been demonstrated that eCBs are closely interconnected to docosahexaenoic acid (DHA) throughout the eicosanoid pathway. Indeed, *N*-docosahexaenoylethanolamine, a derivative of DHA that is thought to be produced by the same pathway that generates AEA, acts as an eCB not only in its ability to activate CB receptors, but also in its susceptibility to be metabolized by the catabolic enzyme fatty acid amide hydrolase (FAAH) (Brown et al., 2010; Cascio, 2013). eCB congeners [i.e., *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA), and *N*-stearoylethanolamine] that exert their effects independently

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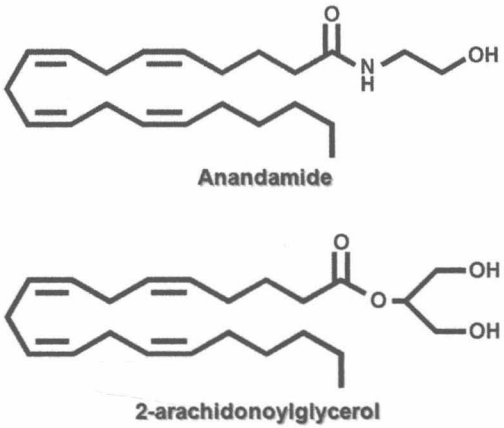

Figure 1.1 Chemical structures of AEA and 2-AG.

of CB receptors are commonly classified as "endocannabinoid-like" compounds (Ben-Shabat et al., 1998; Costa, Comelli, Bettoni, Colleoni, & Giagnoni, 2008; Fezza et al., 2014; Ho, Barrett, & Randall, 2008). Indeed, these molecules might significantly contribute to an entourage effect that prevents true eCBs from being degraded by specific metabolic enzymes, or from allosterically modulating receptor binding (Fezza et al., 2014).

The knowledge of metabolic pathways that regulate the endogenous tone of eCBs, as well as the discovery of the proteins that bind them, shed light on the critical functions of these molecules at central (Maccarrone, Guzmán, Mackie, Doherty, & Harkany, 2014) and peripheral levels (Maccarrone et al., 2015). The most relevant proteins identified to date in controlling eCB tone will be described in the following sections.

N-ARACHIDONOYLETHANOLAMINE METABOLISM: SYNTHESIS

The classical dogma that eCBs are synthesized and released "on demand" via hydrolysis of cell membrane phospholipid precursors has been revisited on the basis of unexpected evidence for intracellular reservoirs and transporters of eCBs. These new entities have been shown to drive intracellular trafficking of eCBs, adding a new dimension to the regulation of their biological activity (Maccarrone, Dainese, & Oddi, 2010). However, several routes have been proposed to explain the metabolic pathways of AEA biosynthesis (Ueda, Tsuboi, & Uyama, 2013).

The possibility to synthesize AEA in vitro by a simple condensation of arachidonic acid (AA) and ethanolamine catalyzed by a reverse FAAH or an AEA hydrolase "working in reverse" was not feasible in vivo (Kurahashi, Ueda, Suzuki, Suzuki, & Yamamoto, 1997; Ueda, Kurahashi, Yamamoto, Yamamoto, & Tokunaga, 1996). Indeed, the substrate concentrations required to form AEA are much higher than those actually detected in cells, and, nowadays, it is accepted that the generation of AEA in cells and tissues occurs mainly as a result of the hydrolysis of a minor membrane phospholipid, *N*-arachidonoylphosphatidylethanolamine (NArPE) (Di Marzo et al., 1994). Although an ever-growing number of enzymes have been ascribed to AEA biosynthesis (Cascio & Marini, 2015), the orchestrated and sequential action of *N*-acyltransferase (NAT) and *N*-acyl-phosphatidylethanolamine (NAPE)-specific phospholipase D (NAPE-PLD) is believed to be the most relevant pathway to generate AEA. Indeed, NAT acts by transferring AA from the *sn*-1 position of 1,2-*sn*-di-arachidonoylphosphatidylcholine (PC) to phosphatidylethanolamine (PE), thus generating NArPE.

The latter compound is next cleaved to yield AEA and phosphatidic acid (PA) by NAPE-PLD. The enzyme is encoded by the *Nape-pld* gene on chromosome 5, position 9.97 cM (21662901–21701396 bp, —strand) (Zimmer, 2015). The transcript has five exons. Exons 2–5 contain the open reading frame of the 396-amino acid NAPE-PLD protein. This 46-kDA protein belongs to the zinc metallohydrolase family of the metallo-β-lactamase fold, is characterized by a highly conserved residues involved in the binding of substrates, is stimulated by divalent cations such as Mg^{2+} and Ca^{2+}, and is involved in the formation of other cannabinoid-receptor-inactive, *N*-acylethanolamines (NAE) (Liu, Tonai, & Ueda, 2002; Okamoto, Morishita, Tsuboi, Tonai, & Ueda, 2004; Petersen & Hansen, 1999; Ueda, Liu, & Yamanaka, 2001). Thanks to the generation of NAPE-PLD-deficient mice, alternative pathways to form NAE in brain have been suggested (Leung et al., 2006; Simon & Cravatt, 2010). Simon and Cravatt (2006, 2008) proposed an NAPE-PLD-independent route, which includes a double-O-deacylation of NAPE via *N*-acyl-lyso-PE and further hydrolysis of the resultant glycerophospho-*N*-acylethanolamine to NAE. This multistep pathway, previously hypothesized by other groups (Natarajan, Schmid, Reddy, & Schmid, 1984; Sun et al., 2004), requires the involvement of a fluorophosphonate-sensitive serine hydrolase and a metal-dependent phosphodiesterase, identified, respectively, as α/β-hydrolase 4 (Abh4) (Simon & Cravatt, 2006) and glycerophosphodiesterase (GDE) 1 (Simon & Cravatt, 2008). Notably, as a lysophospholipase

substrate, Abh4 prefers lyso-NAPE to other lysophospholipids such as lyso-PE, lyso-PC, and lysophosphatidylserine (LPS) (Simon & Cravatt, 2006).

On the other hand, the integral membrane GDE1 possesses Mg^{2+}-dependent phosphodiesterase activity selectively hydrolyzing glycerophosphoinositol (Simon & Cravatt, 2008). Interestingly, double-knockout mice of NAPE-PLD and GDE1 did not exhibit a notable decrease in NAE levels, suggesting the existence of other biochemical routes (Simon & Cravatt, 2010).

Two additional pathways for AEA biosynthesis include (1) sequential hydrolysis of NArPE to phospho-AEA by a phospholipase C (PLC), followed by a dephosphorylation reaction catalyzed by a nonreceptor protein tyrosine phosphatase, identified as PTPN22, to produce AEA (Liu et al., 2006); and (2) hydrolysis of NArPE to *N*-arachidonoyl-lyso-PE (lyso-NArPE), which in turn is converted to AEA by a lyso-PLD (Sun et al., 2004).

Incidentally, it should be mentioned that although the production of NAPE by *N*-acylation of PE is thought to be the rate-limiting step of NAE biosynthesis, the ability to generate the AEA precursor should be equally considered. Several evidence reported that this step might be ascribed also to a calcium-independent NAT, designated as rat LRAT-like protein (RLP-1) or more commonly as iNAT, that removes a fatty acyl group from both the *sn*-1 and *sn*-2 positions (where AA is most abundant) of PC, which is the acyl donor, producing the aforementioned substrate (Jin et al., 2007, 2009).

In the past years, it has been proposed that also *N*-acylated plasmalogen-type ethanolamine phospholipid (*N*-acyl-plasmenylethanolamine, pNAPE) might be converted to NAE through NAPE-PLD-dependent (Petersen, Pedersen, Pickering, Begtrup, & Hansen, 2009; Schmid, Reddy, Natarajan, & Schmid, 1983) and independent pathways (Tsuboi et al., 2011). The latter requires a preliminary deacetylation reaction, probably catalyzed by esterases including phospholipase A_2 ($sPLA_2$)e Abh4, to form a lyso-pNAPE, which is then hydrolyzed to generate NAEs. The identity of the enzyme involved in the last step has to be still ascertained, although the lyso-PLD activity in the brain might be in part attributed to GDE1 (Simon & Cravatt, 2008; Tsuboi et al., 2011). Based on the sequence similarity and the tissue distribution between GDE1 and GDE4, it has been lately suggested that GDE4 might also be responsible for NAE-generating lyso-PLD activities with preferred substrate specificity for *N*-palmitoyl-lysoplasmenylethanolamine (Tsuboi et al., 2015).

It has been reported that five members of the HRAS-like suppressor (HRASLS) family, that are better known as tumor suppressors, possess phospholipid-metabolizing activities including NAPE-forming NAT activity, and it has been proposed to call them HRASLS1–5 PLA/acyltransferase (PLA/AT)-1–5. Among the five members, PLA/AT-1 shows high NAT activity and is mainly expressed in human and murine testis, skeletal muscle, brain, and heart, suggesting this enzyme as mammalian source of NAPE substrates (Uyama et al., 2012, 2013).

N-ARACHIDONOYLETHANOLAMINE METABOLISM: DEGRADATION

The enzymatic breaking down of the amide bond of AEA is mainly ascribed to the action of an FAAH (Cravatt et al., 1996). FAAH is the best characterized enzyme involved in the degradation of AEA and its cloning, crystal structure, kinetic properties, and distribution in the body have been the subject of many interesting reviews (Cravatt & Lichtman, 2003; Fezza, De Simone, Amadio, & Maccarrone, 2008; Maccarrone, 2006; McKinney & Cravatt, 2005).

The *Faah* gene is located on chromosome 4 at position 53,08 cM (115967145–116017926 bp, − strand), has at least 15 exons and produces a transcript of approximately 3.8 kb (Zimmer, 2015). FAAH contains 597 amino acids, corresponding to a molecular weight of ~60 kDa; the optimal pH working value is between 8 and 10, and its distribution in the mammalian brain as well as in peripheral tissues (except for the heart or lung) (Giang & Cravatt, 1997), is overlapping with CB_1 distribution in the same organs (Fezza et al., 2008). This enzyme belongs to a protein family called "amidase signature (AS)," appears as a homodimer bound to membrane lipids via α-helices 18 and 19, exists as a dimer in solution (McKinney & Cravatt, 2005), and is characterized by an unusual serine-serine-lysine (S241-S217-K142) catalytic triad and several channels that allow this enzyme to integrate into cell membranes and establish direct access to the bilayer from its active site (Bracey, Hanson, Masuda, Stevens, & Cravatt, 2002). Circular dichroism and other spectroscopic techniques unveiled the structural features that determine FAAH activity and its membrane-binding properties (Mei et al., 2007). In this context, it has been reported that the structure, subcellular localization, and activity of FAAH are modulated by membrane lipids (Dainese et al., 2014). Indeed, FAAH membrane-binding affinity and enzymatic activity have been shown experimentally to be higher within membranes containing cholesterol and anandamide.