Chapter 9
The Endocannabinoid System: A Dynamic Signalling System at the Crossroads Between Metabolism and Disease

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Abbreviations

(LC-)PUFA	(Long chain-)polyunsaturated fatty acid
2-AG	2-Arachidonoylglycerol
AA	Arachidonic acid
AEA	*N*-arachidonoylethanolamine (anandamide)
CB (receptor)	Cannabinoid (receptor)
CBD	Cannabidiol
COX	Cyclooxygenase
DAGL	Diacylglycerol lipase
DHA	Docosahexaenoic acid (22:6n-3)
DHEA	*N*-docosahexaenoylethanolamine
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
GPCR	G-protein coupled receptor
LOX	Lipooxygenase
MAGL	Monoacylglycerol lipase
NADA	*N*-arachidonoyldopamine
NAEs	*N*-acylethanolamines
OEA	*N*-oleoylethanolamine
PEA	*N*-palmitoylethanolamine
PPAR	Peroxisome proliferator-activated receptor
THCV	Δ9-Tetrahydrocannabivarin
TRVP1	Transient receptor potential channel type V1
Δ9-THC	Δ9-Tetrahydrocannabinol

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1 Introduction: The Changing Views on the Endocannabinoid System

Endocannabinoids are signalling lipids playing important roles in a wide variety of biological processes. Together with their receptors and enzymes involved in their synthesis and breakdown they constitute the "endocannabinoid system" (ECS). By definition the term endocannabinoid is limited to those compounds displaying significant affinity to the cannabinoid receptors CB_1 and CB_2 [1, 2]. These receptors were discovered in the late 1980s [3, 4] and were shown to bind (−)-*trans*-Δ9-tetrahydrocannabinol (Δ9-THC) from the *Cannabis* plant. To date, nine "true" endocannabinoids are being distinguished (Fig. 9.1), which are all derived from long chain (C18 or longer) polyunsaturated fatty acids (LCPUFAs) [1]. The first two discovered and still the most studied are anandamide (*N*-arachidonoylethanolamine (AEA)), its name originating from the Sanskrit word "ananda" meaning "the bliss", and 2-arachidonoylglycerol (2-AG).

However, more than two decades of research have shown that the ECS per se is less specific and distinct than originally assumed. It is now widely accepted that it is tightly intertwined with other signalling mechanisms and that endocannabinoids are part of a larger class of structurally related amides, esters and ethers of fatty acids which exist in a continuous dynamic equilibrium with each other. The vast majority of these molecules belongs to the fatty (acid-) amides like AEA, although analogues of 2-AG including 2-oleoylglycerol and 2-linoleoylglycerol have also been found (see Sect. 2.3). Fatty acid amides (Lipid Maps class FA08; http://www.lipidmaps.org) are conjugates of different long chain fatty acids and amines including ethanolamine, neurotransmitters (serotonin, dopamine) or simple amino acids. They are abundantly present in nature and involved in various regulatory processes. In animals, their molecular targets go far beyond the classical CB receptors and include a wide range of receptors including GPR55, GPR18, GPR119, TRPA1 (transient receptor potential ankyrin 1), TRPV1 (transient receptor potential channel type V1), PPARs (peroxisome proliferator activated receptors) as well as several non-receptor targets [2, 5–7]

It has also become clear that some (if not all) of the "true" endocannabinoids themselves display "promiscuous" behaviour by activating or blocking other receptors besides CB_1 or CB_2 with potencies that differ little from those with which they interact with "true" cannabinoid receptors [2, 6]. In addition, anandamide, 2-AG and other CB ligands interact directly or indirectly with non-receptor targets [5]. Biochemical pathways for synthesis and degradation of endocannabinoids and their congeners show several crossroads with those of other lipid mediators, in particular eicosanoids. This not only creates a number of regulatory nodes but also leads to the formation of "hybrid" structures including prostamides and other oxidation products, often with bioactivity [8–11]. Taken together, an "expanded" view of the ECS is increasingly considered a better concept to comprehend its full dimensions [12]. In line with this, it has been suggested to apply the term "endocannabinoidome" to describe this family of molecules (Fig. 9.2). Mediators that are part of this endocannabinoidome are fluctuating in a time and tissue-specific way, modulated by various endogenous (e.g. energy status, inflammation) and

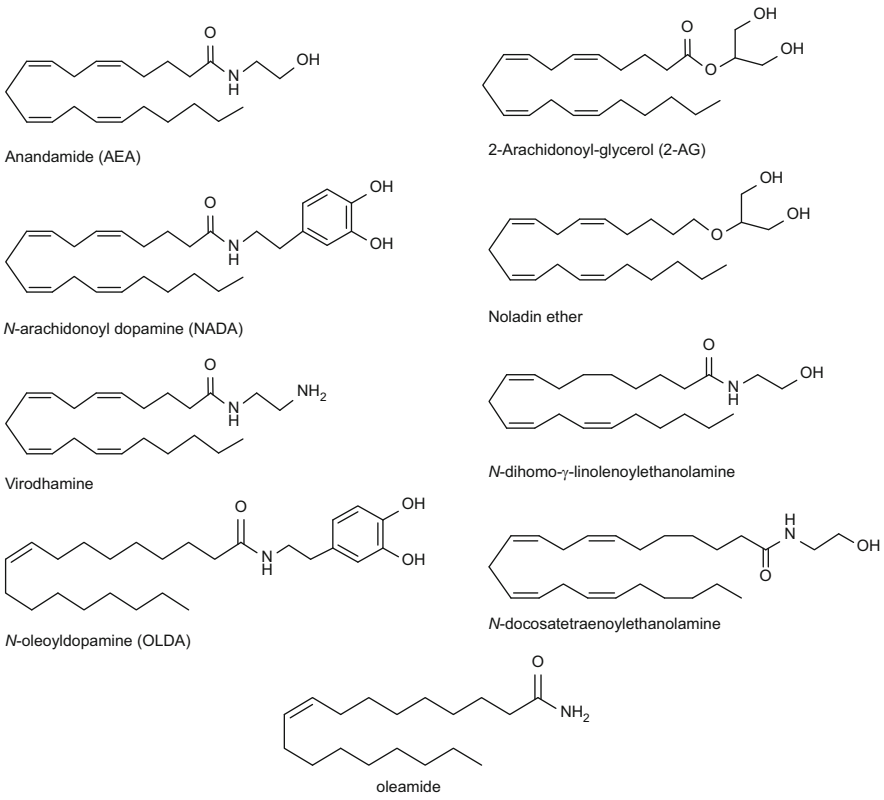

Fig. 9.1 Structural formulas of the classical endogenous cannabinoid receptor agonists, anandamide (*N*-arachidonoylethanolamine, AEA), 2-arachidonoyl glycerol (2-AG), *N*-arachidonoyl-dopamine (NADA), Noladin ether, *O*-arachidonoylethanolamine (Virodhamine), *N*-dihomo-γ-linolenoylethanolamine, *N*-oleoyldopamine (OLDA), *N*-docosatetraenoylethanolamine and oleamide

environmental factors, including diet. These network dynamics have major consequences for drug development. Soon after its discovery it became clear that the ECS is involved in a number of important processes and (chronic) diseases including pain, anxiety/depression, GI/liver diseases, cancer, metabolic disease and eating behaviour. Several promising new pharmacological targets were suggested which often indeed showed encouraging effects in animal studies. In particular in relation to weight management and metabolic diseases expectations were high to develop CB_1 antagonists or inverse agonists into a completely new drug class. Therefore, the failure of the first in class compound rimonabant because of severe anxiety and depression-related side-effects in predisposed persons [13] shocked the pharmaceutical industry. By the end of 2008 at least nine companies terminated active development projects with CB_1 blockers. These included some with compounds in a well-advanced stage of development such as Taranabant (Merck) and CP-945,598 (Otanabant, Pfizer). In retrospect these failures illustrate that initial

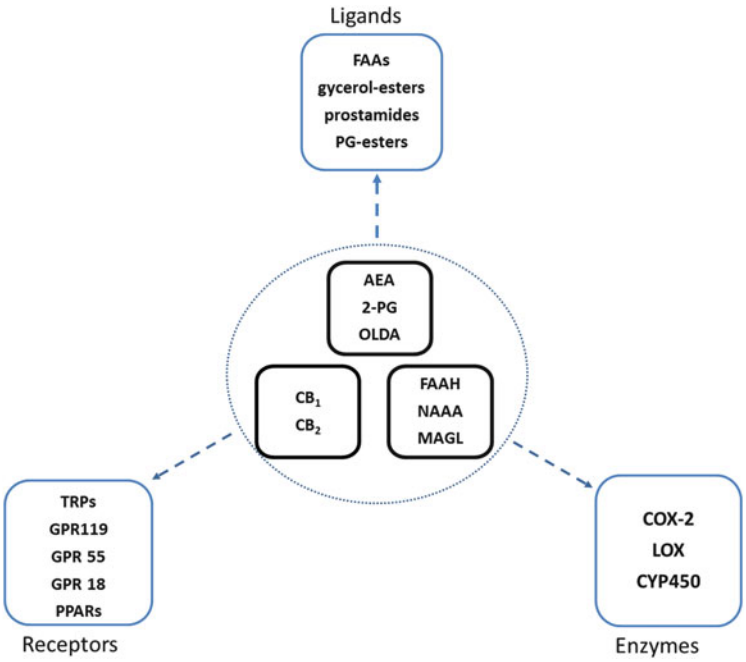

Fig. 9.2 Cartoon depicting the "expanding" view on the endocannabinoid system (ECS). The "classical" ECS (*centre*) is considered as part of an *endocannabinoidome* consisting of structurally related ligands, metabolites and enzymes involved. Endocannabinoids per se and their congeners interact with different non-cannabinoid receptors and other molecular targets

strategies to modulate a dynamic and pleiotropic like the ECS have been too narrow. The endocannabinoidome still holds many promises for both "food" and "pharmaceutical" applications. However, its complexity demands for more subtle multiple-target strategies instead of a classical one disease–one target–one drug approach. This chapter aims to illustrate some recent developments and activities in the field of the ECS, including some related receptors and mediators. Major therapeutic applications will be briefly illustrated. This will be elaborated in Sect. 4 by examples from two main domains, namely, "inflammation" and "weight management".

2 From Phytocannabinoids to Endocannabinoids: A classical Example of Reversed Pharmacology

2.1 Compounds with Pharmacological Activity from Cannabis spp.

Earliest written records on the physiological effects and medical use of *Cannabis* go back to about 2000 BC in the famous book Pe'n-ts'ao Ching attributed to the

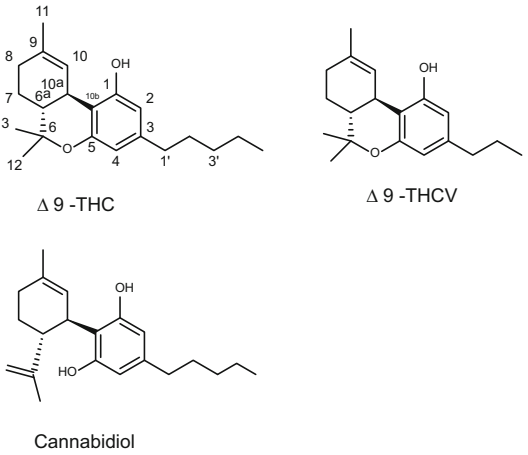

Fig. 9.3 Examples of phytocannabinoids from *Cannabis*. The main psychoactive component is (−)-*trans*-Δ9-tetrahydrocannabinol (Δ9-THC). Two other compounds discussed in this chapter are (−)-cannabidiol (CBD) and (−)-*trans*-Δ9-tetrahydrocannabivarin (Δ9-THCV)

Chinese emperor Shen-nung [14]. This ancient pharmacopoeia describes a number of properties of *Cannabis*, including its capacity to "lighten one's body". Throughout history, medical use of *Cannabis* has been widely accepted and very common in different parts of the world until this began to decline around the beginning of the 1900s [15]. Since the last decades, there is a renewed interest in preparations and compounds prepared from the *Cannabis* plant. The term *phytocannabinoids* (phyto- used here to distinguish them from endocannabinoids) refers to a group of terpenophenolic compounds with 22 carbons (or 21 carbons for neutral form) of which more than 70 have been found so far and which can be categorised into ten main structural types [16, 17]. In general, *Cannabis* refers to the species *Cannabis sativa*, although there is ongoing discussion whether the genus *Cannabis* comprises more than one species, i.e. *Cannabis sativa* and *C. indica* [16]. Preparations from different *Cannabis* breeds show a great variety in absolute and relative concentrations of phytocannabinoids [18], of which only a few are ligands for CB_1 or CB_2 receptors. However, as the adjective "cannabinoid" predates the discovery of cannabinoid receptors by many years this term is still commonly used to describe also other compounds with structures similar to the phytocannabinoid Δ9-THC, irrespective of whether they are or are not cannabinoid receptor agonists or antagonists [2]. Recent breeding and selection of *Cannabis* for recreational purposes has primarily focussed on increasing the content of the psychotropic compound (−)-*trans*-Δ9-tetrahydrocannabinol (Δ9-THC, Fig. 9.3). At the same time, the renewed interest in *Cannabis* for medical use initiated the search for cultivars with completely different compositions and often much lower hallucinogenic activity. It is expected that the unravelling of the *Cannabis sativa* genome [19] will further stimulate these developments. In the plant, cannabinoids are produced as their carboxylic acid derivatives, known as cannabinoid acids. Their neutral counterparts can be formed through the action of heat (smoking), sunlight or during storage [20, 21]. Several cannabinoid acids themselves display biological activity, which are often distinct from those of their decarboxylated products [17, 20–22]. Chemical structures of some of the most studied phytocannabinoids are depicted in Fig. 9.3.

Although Δ9-THC (Dronabinol, Marinol®) has been available as medicinal preparation for oral use since the 1980s, its therapeutic use initially remained rather limited. Efficacy was reported to be variable, at least partly due to significant first-pass metabolism [23]. Since the beginning of this century there has been a slow but steady growth in the development and application of medicinal products based on herbal *Cannabis* or natural cannabinoids [23–25]. Differences between regions and therapeutic viewpoints are among the factors which determine whether the focus is more on the herb or on specific phytocannabinoids. Some countries and regions (The Netherlands, Canada, several US states) have official medicinal *Cannabis* policies, often referred to as "medical marijuana". Herbal products are preferably taken by inhalation using special vaporizers, and there is an increasing trend towards "individualised" therapies using specially selected cultivars [18]. On the other hand, formulations with purified Δ9-THC, CBD and/or THCV for oral or oromucosal delivery are also being developed and implemented [26, 27]. *Cannabis* or phytocannabinoid-based preparations are used for a number of indications, including pain, the amelioration of chemotherapy-induced nausea and vomiting, stimulation of appetite and management of spasticity in multiple sclerosis [25, 27, 28]. An in-depth discussion on the role of *Cannabis* or cannabinoid-based preparations in medical therapy is considered outside the scope of this chapter. It is obvious though that the debate on this issue continues until today.

From a scientific and potentially therapeutic perspective it is of interest to note that increasing data are becoming available on the activity of phytocannabinoids other than Δ9-THC with only weak or no psychotropic effects. Compounds of interest include cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), Δ9-tetrahydrocannabivarin (THCV), cannabidivarin (CBDV) as well as (at least) some cannabinoid acids, Δ9-tetrahydrocannabinolic acid (Δ9-THCA) and cannabidiolic acid (CBDA). A detailed discussion of each of these molecules falls outside the scope of this chapter. For an excellent overview readers are referred to Izzo et al. [17]. However, two compounds are of specific interest and merit some extra attention here, namely, CBD and THCV.

Among the non-hallucinogenic phytocannabinoids, CBD (Fig. 9.3) is currently receiving the most attention. In dried *Cannabis* CBD contents range from very low (<1 %) to equal or even higher (up to around 8 %) compared to those of Δ9-THC, depending on the cultivar and preparation. The oromucosal spray Sativex®, prescribed for the treatment of spasticity due to multiple sclerosis contains CBD and Δ9-THC in a 1:1 ratio. Cannabidiol behaves like a typical multiple target compound. For reviews, see for example [17, 29]. It displays a highly diverse spectrum of activities including agonist activity for PPARγ, TRVP1 and TRPA1 receptors, antagonist of GPR55, a complex antagonistic behaviour towards CB_1 and CB_2, etc. (see also Sect. 3.2). Specifically in relation to neurodegenerative diseases and (neuropathic) pain the effects of CBD on glia cells are of interest to note. Several preclinical and an increasing number of clinical studies have suggested at least promising activities in chronic inflammatory and autoimmune diseases including IBD [30], MS [31], cancer [5, 32, 33] and different CNS disorders [34–36]. Remarkably, there is increasing evidence that CBD and Δ9-THC interact within the CNS

thereby reducing the psychoactive effects of Δ9-THC and possibly even its psychogenic risks [37–39]. Future clinical studies, of which several are presently ongoing (source: ClinicalTrials.gov) should further demonstrate the full therapeutic potential of CBD, alone or combined with other cannabinoids or other compounds.

Δ9-tetrahydrocannabivarin (THCV, Fig. 9.3) occurs in *Cannabis* as a minor component in varying amounts [18]. Interestingly, this compound has been found to possess CB_1 antagonist properties [40, 41]. Therefore, it is receiving attention as a natural alternative to the CB_1 blockers/inverse agonists like rimonabant. Recently, THCV has been found to ameliorate insulin sensitivity in two mouse models of obesity [42].

2.2 *Phytocannabinoids from Plants Other than* Cannabis

Remarkably, structures with affinity to CB_1 and CB_2 have also been found in plants other than *Cannabis* [43]. These molecules may be divided into two categories. First, plants like most other organisms contain lipid-derived structures which are chemically related to the endocannabinoids as those found in mammals (see also Sect. 2.3), albeit shorter acyl chains (C12 or C14) appear to be more common in plant [43–46]. Next to this, an increasing number of other plant compounds with affinity for CB_2 or CB_1 have been characterised. Examples include (E)-ß-caryophyllene (present in many different spices and food plants including oregano, cinnamon and black pepper), falcarinol (found in carrots, parsley and celery), yangonin (present in Kava, *Piper methysticum*) and magnolol (from the medicinal plant *Magnolia officinalis*) (Fig. 9.4) [43, 44, 47–50].

Considering the wide abundance in nature and "promiscuity" of the ECS and related signalling systems, it does not seem unlikely that more natural compounds with similar properties will be found in common spices and herbs. It is tempting to speculate that such compounds may play a role in the culinary properties of some plants by inducing "hedonic" signals in the brain via CB_1 receptor stimulation.

2.3 *Endocannabinoids and Beyond*

As mentioned in Sect. 1, the discovery of the prototypical endocannabinoids per se was followed by the finding that these molecules belong to much larger group of fatty acid-derived structures of which the biological effects go far beyond effects on CB_1 and CB_2.

The endocannabinoid anandamide (AEA) belongs to *N*-acylethanolamine (NAE) subclass of fatty acid amides. In addition to the NAEs, several other classes of fatty acid amides can be distinguished, including the primary fatty acid amides, the *N*-acylamino acids (=*N*-acylamines), *N*-acylarylalkylamines (*N*-acyldopamines, *N*-acylserotonins) (Fig. 9.5) [51, 52].

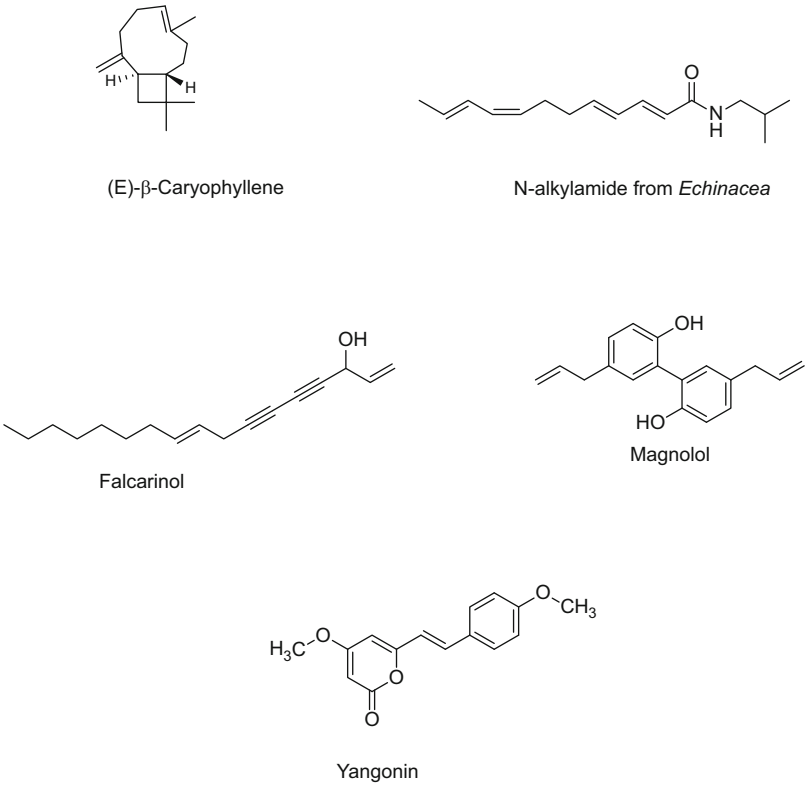

Fig. 9.4 Some plant-derived compounds with CB_1 or (and) CB_2 affinity present in plants other than *Cannabis*

It has been shown that cells are able to "combine" different fatty acids and biogenic amines to make several possible permutations of different fatty acid amides [7, 51]. Several studies have demonstrated that the local relative availability of fatty acid precursors, which in turn is modulated by dietary intake of lipids, plays an important role in determining the pattern of amide conjugates formed. For example, a number of studies in rodents and humans have shown that increasing the relative proportion of n-3 LC PUFAs in the diet can lead to a decrease in the formation of the "prototypic" endocannabinoids AEA and 2-AG, which are derived from the n-6 fatty acid arachidonic acid [53–56]. These changes are a direct consequence from a shift in n-3–n-6 balance of membrane lipids, resulting in compensatory increases in n-3 LC-PUFA-derived acyl conjugates. The same holds true for the local availability of amines. For example, we showed that serotonin conjugates with fatty acids are formed by gut tissue, where most of the body's serotonin resides [57].

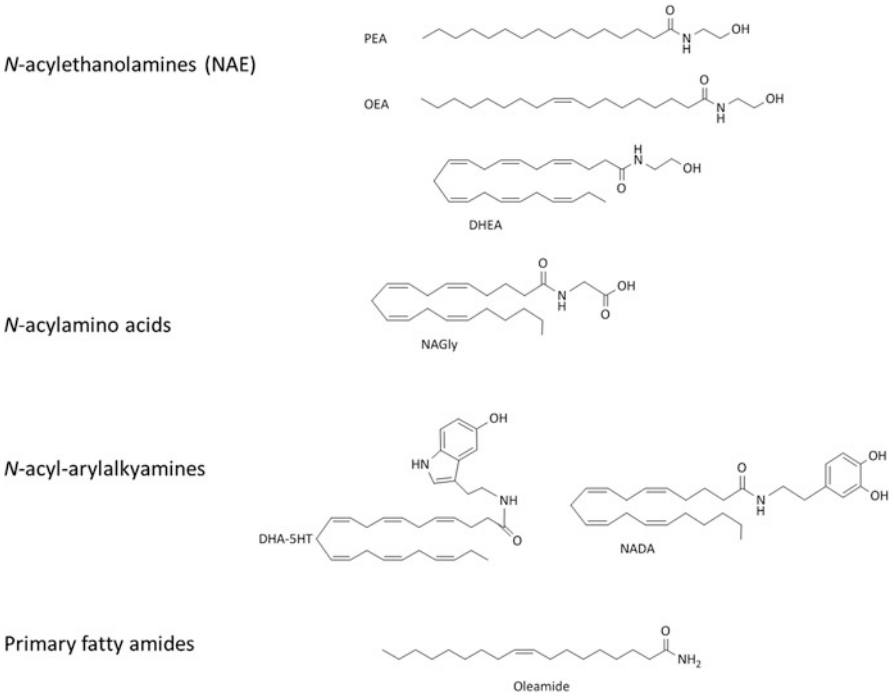

Fig. 9.5 Examples of fatty acid amide structures not belonging to the endocannabinoids per se (see also Fig. 9.1)

Compared to the many fatty acid amides, only little has been reported on 2-acylglycerol esters other than the endocannabinoid 2-AG. However, it is likely that several congeners will exist, for example formed out of triglycerides. In 1999, Ben-Shabat et al. reported the isolation of 2-linoleoyl-glycerol and 2-palmitoyl-glycerol (2-PG) from mouse spleen, brain and gut [58]. These two compounds did not directly bind to CB_1 or CB_2 receptors but apparently potentiated the binding of 2-AG and its capacity to inhibit adenylyl cyclase. Furthermore, both esters caused potentiation of some of the in vivo effects of 2-AG. Interestingly, 2-oleolyglycerol (2-OG) was found to stimulate GPR119 receptors (see Sect. 3.2.4) in vitro, and did this more potently than 2-AG, 2-PG and 2-linoleoyl-glycerol [59]. Subsequently 2-OG was given to human volunteers (2 g by jejunal delivery), which resulted in increased levels of plasma GLP-1 compared to administration of the precursor oleic acid. Triglycerides with oleic acid in the *sn*-2 position are very common in the diet and from these 2-OG can be formed in the gut in amounts larger than the dose given in the study of Hansen et al. [59]. Very recently, the presence of 2-linoleoyl-glycerol and 2-oleoylglycerol (2-OG) has also been demonstrated in *Drosophila* [60].

3 Biochemistry and Pharmacology of the ECS

3.1 Endocannabinoid Formation and Breakdown

Synthesis and release of endocannabinoids and many related compounds are considered to take place "on demand" via different multi-step processes which are partly acting in parallel. For *N*-acyl-ethanolamines (NAEs) the most studied pathway is their formation from glycerophospholipids via *N*-acylphosphatidyl ethanolamines (NAPEs), present in phospholipid membranes. NAPEs function as stable precursors and source of their respective NAEs. Besides their role as precursor of NAEs, NAPEs have bioactive effects themselves [61]. NAPEs are formed by incorporation of fatty acids from the *sn*-1 position of a donor phospholipid like phosphatidylcholine and transfer to an ethanolamine phospholipid, e.g. phosphatidylethanolamine. This reaction is catalysed by a Ca^{2+}-dependent *N*-acyltransferase [61, 62]. Next, free NAE can be generated by a NAPE-hydrolyzing phospholipase D (NAPE-PLD). In addition, other synthesis routes for NAEs have been found [61, 63]. The glycerol-ester 2-AG is synthesised from diacylglycerol (DAG), a very common second messenger, via the enzyme diacylglycerol lipase (DAGL), of which more than one form has been described [64]. Biosynthetic routes for other *N*-acylamides appear to be less well studied [52]. Huang et al. originally suggested that *N*-arachidonoyldopamine (NADA) may either be synthesised by condensation of dopamine with arachidonic acid (possibly via arachidonoyl-CoA) or via a pathway involving *N*-arachidonoyl-tyrosine [65]. Later, Hu et al. [66] reported that the latter may not be very likely. Instead they suggest a direct involvement of FAAH either as rate-limiting enzyme that liberates arachidonic acid from AEA, as a conjugation-enzyme, or both.

Conjugates of arachidonic acid (and possibly other fatty acids) with simple amino acids can be synthesised via the formation of the acyl-CoA thioesters, as was shown for *N*-arachidonoyl-glycine (NAGly) [67]. Interestingly, the arachidonic acid that conjugates with glycine appears to be a result of the hydrolysis of AEA [68]. An alternative pathway was proposed by Burstein et al. [69] who speculated that NAGly is produced by the oxidation of the ethanolamine in AEA, presumably through alcohol dehydrogenase. Evidence exists for both pathways [68].

Fatty amides and 2-acylglycerols are broken down by enzymatic hydrolysis. The primary NAE degrading enzyme is fatty acid amide hydrolase (FAAH, now also known as FAAH-1), localised on the endoplasmatic reticulum [70]. A second FAAH enzyme is found in humans located on cytoplasmic lipid droplets [70, 71]. Recently, a third NAE hydrolysing enzyme, *N*-acyl ethanolamine-hydrolysing acid amidase (NAAA) has been identified [72].

To reach their sites of catabolism within the cell, NEAs are bound to different proteins including fatty acid binding proteins 5 and 7, heat shock protein 70, albumin and fatty acid amide hydrolase-like AEA transporter protein [73, 74]. Intracellular trafficking of NAEs is also important to reach those receptors that are located intracellularly [55, 75]. Next to hydrolysis, NAEs are substrates for oxidative

enzymes including cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 enzymes, yielding a range of prostaglandin-amides (prostamides), prostaglandin-glycerol esters and hydroperoxy-derivatives [5, 76, 77]. At least a number of these oxidation products show biological activity [76–78]. 2-AG is hydrolyzed via the enzyme monoacylglycerol lipase (MAGL), to a lesser extent by α/ß-hydrolase 12 (ABHD12) and α/ß-hydrolase 6 (ABHD6), and also by FAAH [79, 80]. Interestingly, AA in brain formed by hydrolysis of 2-AG via MAGL has been shown to serve as pool for pro-inflammatory eicosanoid synthesis, thus representing a connection between endocannabinoid and eicosanoid pathways [81]. 2-AG can also be oxygenated by COX-2 and LOX resulting in the formation of prostaglandin glycerol esters (PG-Gs) [76].

3.2 Cannabinoid and Related Receptors

According to the IUPHAR classification system the CB_1 and CB_2 receptors are the only bona fide cannabinoid receptors. They are phylogenetically restricted to the chordate branch of the animal kingdom [2]. Among other GPCRs, those structurally most closely related to CB_1 and CB_2 belong to the lysophospholipid receptors. These receptors for endocannabinoids or lysophospholipid-like molecules have evolved independently in different branches of the GPCR superfamily [1]. However, in terms of ligand binding characteristics the picture becomes more complicated. As mentioned before, endocannabinoids have a multitude of structural analogues. These compounds interact with different receptors and non-receptor targets. Several endocannabinoids per se, including anandamide, but also Δ9-THC and a number of synthetic CB_1 or CB_2 agonists and antagonists can activate or block different non-cannabinoid receptors with potencies that differ little from those with which they activate or block the "true" cannabinoid receptors [1]. According to nomenclature criteria of the NC-IUPHAR cannabinoid receptor subcommittee the TRPV1 channel might eventually come to be regarded as being either an "ionotropic cannabinoid CB_3 receptor" or a dual TRPV1/CB_3 receptor. In addition, some other receptors deserve further attention in this respect, namely, GPR18, GPR55, GPR119 and the peroxisome proliferator-activated receptors (PPARs) α and γ. Although these show little to no structural similarity to CB_1 and CB_2 they have been shown to respond to endocannabinoids, their endogenously present congeners and (or) plant-derived "phyto"-cannabinoids.

3.2.1 CB_1 Receptors

CB_1 receptors are presynaptically located at central or peripheral nerve terminals and act as modulators of synaptic transmission by a process which has been called retrograde signalling {Wilson, 2002 #4848; Cachope, 2012 #3683; Vaughan, 2005 #4853}. Physiological stimulation of neurons induces the synthesis of

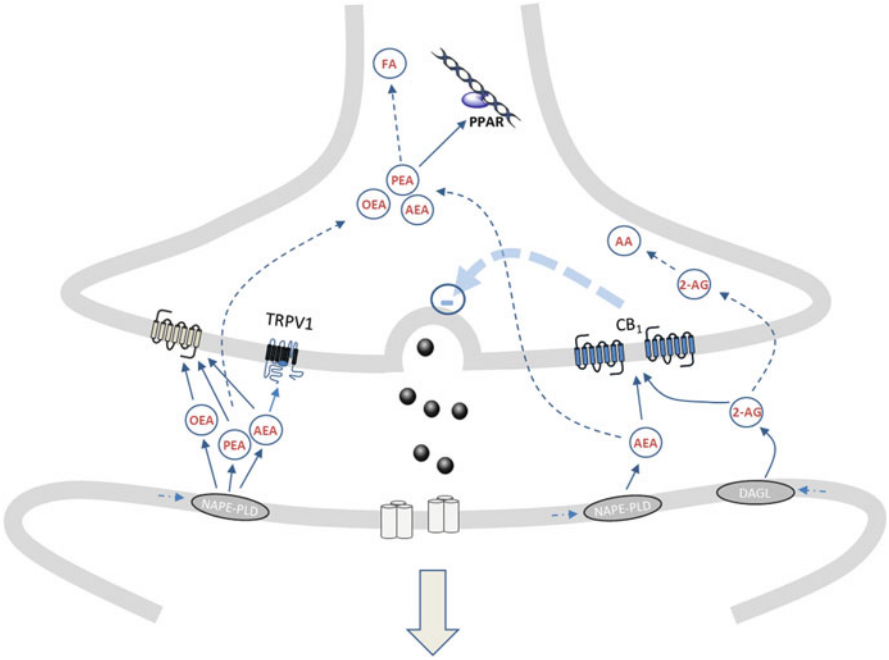

Fig. 9.6 Schematic representation of the mechanism of retrograde signalling by endocannabinoids at a synaptic cleft. Neuronal depolarization causes cleavage of membrane lipid precursors to induce de novo synthesis and release of endocannabinoids such as AEA, PEA, OEA and 2-AG into the synaptic cleft. These endocannabinoids activate cannabinoid CB_1 receptors located on presynaptic terminals of neurons which reduces release of neurotransmitters (such as GABA or glutamate) onto the postsynaptic neuron. Endogenously released cannabinoids might also act via TRP ligand gated ion channels (e.g. TPRV1) and other GPCRs (e.g. GPR 119). Endocannabinoids are taken back up into neuronal and glial cells and then degraded by enzymes such as fatty acid amide hydrolase (FAAH) and MAG-lipase (MAGL)

endocannabinoids in the post-synaptic nerve terminal and this reduces synaptic inputs in a highly selective and restricted manner (Fig. 9.6).

The majority of CB_1 receptors are coupled through $G_{i/o}$ proteins. Their stimulation leads to inhibition of adenylate cyclase activity, effects on different Ca^{2+} and K^+ channels, and stimulation of mitogen-activated protein (MAP) kinase. In some cases CB_1 receptors signal through G_s proteins [1, 2, 80].

In contrast to what was originally assumed, the distribution of CB_1 receptors is not limited to the CNS, and CB_1 receptors are also found in the immune system, vascular endothelium, intestine, liver, skeletal muscles, peripheral nerve synapses and reproductive tissues. As a consequence of their localisation at the terminals of central and peripheral neurons where they mediate inhibition of neurotransmitter release, CB_1 receptors are involved in a wide variety of biological processes [1] including learning and memory, anxiety, pain, eating behaviour, metabolism, reproduction and growth and development. As a result they have been associated

with different disorders and diseases (Sect. 4). For example, their involvement in food intake regulation (Sect. 4.2) takes place at different levels, starting from receptors within the GI tract to the regulation of hedonic rewarding in the brain [55, 82–84]. Its presence in peripheral tissues also provides an explanation for the sustained effects of the CB_1 inverse agonist rimonabant on body weight and the improvement of insulin resistance and blood lipids, in addition to its short-term appetite-decreasing effect. On Vagal afferents CB_1 expression was found to be regulated by CCK [85] and high/low fat diets [86]. Remarkably, peripheral stimulation of CB_1 receptors on Vagal afferents by anandamide was shown to reduce appetite, whereas central stimulation of CB_1 receptors increased food intake [87]. In the brain, the CB_1 is now regarded the most abundant G-protein coupled receptor [2]. A pioneering study on its distribution in brain was published in 1990 by Miles Herkenbaum et al. [88]. More recent reviews include the following references [89, 90]. As mentioned before, the central regulation of energy intake and metabolism is one of the major functions of the "classical" ECS. Within the brain, CB_1 receptors have been linked to several both homeostatic and non-homeostatic regulation mechanisms, with endocannabinoids acting as modulators of orexigenic and anorexigenic neurotransmitters and neuropeptides by presynaptic regulation of their release. The brain ECS shows numerous anatomical and functional connections with other signalling pathways including dopaminergic, opioid and GABA-ergic systems involved in pleasure and reward, pain, anxiety, fear, etc. [55, 91–95].

3.2.2 CB_2 Receptors

CB_2 receptors are predominantly expressed on immune and haematopoietic cells, but functionally relevant expression has also been found in specific regions of the brain, other tissues and in various tumours. Like CB_1 receptors they are coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase activity and positively to MAP kinase. Although several studies have suggested that CB_2 activation is immunomodulatory and neuroprotective [96–98], some data remain inconclusive. This may be partly due to the fact that different components of the inflammatory cascade can be affected in a different direction [99]. Furthermore, discrepancies are caused by the use of different animal models, compounds and doses [100]. Disease-induced changes (usually increases) in CB_2 receptor expression have been reported [101]. Furthermore, many synthetic CB_2 receptor agonists have shown protective effects in a variety of preclinical disease models and pathological conditions (reviewed by ref. 101). Therefore, the application of selective CB_2 agonists would be of interest for a number of disorders (Review: [28]). At the same time the wide abundance of CB_2 receptors and the critical importance of retaining an adequate pro-inflammatory balance present challenges for their application as therapeutic targets [101]. Therefore, subtle and well-balanced approaches, including multiple targeted and/or localised therapies are likely to provide the best options [29].

3.2.3 Transient Receptor Potential (TRP) Cation Channels

Transient receptor potential (TRP) cation channels constitute a superfamily of receptors involved in the signal transduction of a wide range of stimuli, including effects elicited by endogenous lipids [2, 102–104]. Mammalian TRPs are subdivided into six protein families of which three are here considered of particular relevance because they bind endocannabinoids and related compounds and (or) phytocannabinoids. These are: the vanilloid receptor TRPs (TRPVs, in particular TRVP1), the melastatin or long TRPs (TRPMs, in particular TRPM8) and ankyrin transmembrane protein 1 (TRPA1).

The *TRVP1* receptor is particularly known as the receptor for the vanilloid capsaicin present in red peppers. In addition it is perhaps the best established non-cannabinoid receptor for endocannabinoids, and for anandamide in particular [7, 95]. Several papers note the overlap between the ECS and what has been called "endovanilloid system" [95, 105–107]. Based on this it has been suggested to rename the TRVP1 receptor to "ionotropic cannabinoid CB_3 receptor" or a dual TRPV1/CB_3 receptor (see also Sect. 3.2). *N*-arachidonoyl-dopamine (NADA) was the first fatty acid amide shown to act as endogenous ligand of TRVP1 receptors [65]. Meanwhile several other *N*-acyl amides have also been demonstrated to activate TRPV1 [51]. TRVP1 is predominantly expressed in sensory neurons but also on non-neuronal cells including epithelial, endothelial and smooth muscle cells as well as in lymphocytes, hepatocytes and pancreatic cells [2, 5, 108]. Historically, TRPV1 has been considered a pro-inflammatory receptor in several conditions, including neuropathic pain, joint inflammation and inflammatory bowel disease. A number of TRVP1 antagonists have been developed as potential drugs against different forms of pain, but so far results in the clinic were not successful [108]. Recent evidence also demonstrates paradoxical, protective functions of TRVP1 in vivo [109]. The receptor also plays a role in energy metabolism and weight management as recently reviewed by Ahern [102]. For example, there is long-standing evidence that dietary consumption of chilli peppers can affect body weight. Treatment with capsaicin, or related "vanilloid" compounds, reduces weight gain and adiposity in animals consuming moderate to high-fat diets. An interesting finding was that the endogenous endocannabinoid congener *N*-arachidonoyl-serotonin (AA-5-HT) displays dual activity as both FAAH inhibitor and TRPV1 antagonist. The compound has shown marked effects against both acute and chronic peripheral pain in rodent models [110, 111]. Previous studies from our lab showed that this conjugate is particularly present in the gut, but so far its biological role has not been established [57]. In addition to TRPV1, other members of this family, including TRPV2-4 have been associated with, in particular effects of phytocannabinoids and (or) *Cannabis* extracts [2, 5, 112].

The *TRPM8* receptor is involved in the detection of sensations such as cold. Activators include eucalyptol, menthol and icilin [113]. It is considered a therapeutic target for cold hypersensitivity and neuropathic pain [108]. Its expression was also found to be important for the survival of androgen receptor-dependent

prostate cancer cells [5]. Both anandamide and *N*-arachidonoyl dopamine, but not 2-AG, were shown to antagonise the stimulatory effect of menthol and icilin on TRPM8 [114]. In addition, several phytocannabinoids show activity on TRPM8 [112, 114].

The *TRPA1* receptor is receiving increasing attention as a key regulator of neuropeptide release, neurogenic inflammation and pain. See [108, 115] for reviews. TRPA1 was found to be activated by CBD [114]. Another phytocannabinoid, cannabichromene can also act as TRPA1 agonist. The receptor was shown to be involved in the inhibition of nitric oxide production in macrophages and the amelioration of murine colitis by cannabichromene [116].

3.2.4 GPR119

GPR119 ([117] for a recent review) has been described as a class A (rhodopsin-type) orphan GPCR but has no close primary sequence relative in the human genome. Two of its endogenous ligands discovered so far are the fatty acid amides oleoylethanolamide (OEA) and *N*-oleoyldopamine (OLDA). Furthermore the receptor can be activated, albeit with less potency, by PEA, EAE and linoleylethanolamine (LEA) [2, 118, 119]. As none of these compounds are ligands for CB_1 or CB_2 receptors, GPR119 is not considered a cannabinoid receptor per se [2]. Recently, GPR119 has also been found to respond to 2-oleoyl-glycerol, a compound formed out of common dietary triglycerides (described in Sect. 2.3). Following its de-orphanization in 2006 by Overton et al. [120] and the demonstration that small molecule agonists are able to reduce body weight gain in rodents, GPR119 has attracted considerable attention. The receptor is Gαs-protein coupled and predominantly expressed in pancreatic islets and gastrointestinal tract in humans and rodents. GPR119 agonists were found to increase intracellular cAMP, which in turn leads to increased GLP-1 secretion from entero-endocrine cells. Following the synthesis of the first ligands, including PSN632408 and AR-231,453 several pharmaceutical companies became active in developing GPR119 agonists. Many of these compounds have shown interesting activities in animal models of type 2 diabetes and obesity, including a reduction of blood glucose without causing hypoglycaemia, a reduction of food intake and body weight, and reduced diabetes progression. Presently, a number of GPR119 agonists are in advanced stages of development [121].

3.2.5 GPR 55

The discovery of the orphan GPCR GPR55 was first described in 2007 [122]. The receptor was shown to bind some CB_1 and CB_2 ligands. Therefore, it has been considered a "novel" or "third" cannabinoid receptor for some time, but this viewpoint has been abandoned. Structurally the receptor has no significant sequence similarity with the CB receptors, in particular not in the areas responsible for ligand binding [2]. GPR55 is expressed in the gut and found in cells of the

immune system, including microglia in brain as well as in endothelial cells [123]. A recent study suggested that GPR55 regulates CB_2 function in human neutrophils [124]. Following the report of Oka et al. [125] it is now assumed that its endogenous ligand is lysophosphatidylinositol (LPI). It was suggested by Ross [126] that LPI and GPR55 might play a role in driving cancer cell proliferation and migration. The phytocannabinoid CBD shows antagonist activity towards GPR55, which may of therapeutic relevance [127].

3.2.6 GPR18

The GPR18 gene was first cloned in 1997 [128] and at that time found to be highly expressed in human testis and spleen. In addition, its presence was shown in thymus, peripheral white blood cells and in the small intestine, whereas in many other tissues and organs it appeared to be absent. McHugh et al. [129] demonstrated that NaGly (*N*-arachidonoylglycine, see Sect. 3.1) serves as an endogenous ligand. The same group also reported that two cannabinoid agonists, AEA and THC, are full agonists at GPR18, whereas CBD displays low efficacy as agonist [130]. Considering its location on microglia cells [131] and on peripheral macrophages, GPR18 and its endogenous ligand(s) are receiving increasing attention in relation to inflammation.

3.2.7 Peroxisome Proliferator-Activated Receptors (PPARs)

PPARs can be activated by some non-cannabinoid NAEs including OEA and PEA. The same has been shown for some 2-AG derivatives of the COX/LOX/CYPP450 pathways, and to a lesser extent also for AEA and 2-AG itself. PPARs are ligand-activated transcription factors that play critical roles in very different biological pathways such as lipid, protein, glycerol, urea, glucose, glycogen, and lipoprotein metabolism, adipogenesis, trophoblast differentiation and cell migration. For recent reviews see for example [132, 133]. Their best known agonists are various fatty acids and their derivatives. Therefore, PPARs are commonly regarded as general—not very selective—lipid sensors monitoring local metabolic changes. The PPAR family consists of PPARα, PPARβ and PPARγ. The three PPAR iso-types are similar in homology, but show their own distribution pattern. In humans PPARα is localised in areas of high fatty acid catabolism (kidneys, liver, heart, brown adipose tissue and intestines). PPARγ is found as two isoforms: PPARγ1 (predominantly present in gut, brain, vascular cells and immune cells) and PPARγ2 (mainly in adipose tissue). PPARβ/δ has been found in many tissues and is particularly highly active in skeletal muscle, smooth muscle and skin [132].

The role of the PPARα receptor as a pivotal switch in different inflammatory and pain signalling pathways in the CNS and periphery is widely acknowledged [132, 134, 135]. Two well-known *N*-acylamides that are linked to this PPAR are PEA and OEA. For PEA (see also Sect. 4.3.2) it is assumed that its anti-inflammatory activity

can largely be assigned to an agonist activity on PPARα [135–137]. PPARα is also playing a pivotal role in the satiety-inducing effects of OEA [138]. This NAE is formed form oleic acid in the epithelium of the proximal small intestine. PPARγ serves as the molecular target for the thiazolidinediones, an important class of anti-diabetic drugs. Its major natural ligands and activators are PUFAs and fatty acid-derived molecules. The beneficial action of PPARγ has typically been attributed to increased insulin sensitivity and reduced inflammation. Agonism of PPARγ is increasingly considered an important property of the phytocannabinoid CBD (Sect. 2.1). PPARγ and CBD are also receiving attention in relation to CNS diseases like Alzheimers' disease because of the role of PPARγ in stimulating microglial function [139, 140].

3.3 Interactions of Endocannabinoids with Non-receptor Targets

Several studies suggest that the biological activities of at least some of the endocannabinoids and their congeners are not exclusively mediated through GPCRs or nuclear receptors. An example comes from the anti-inflammatory effects of *N*-docosahexaenoylethanolamine (DHEA, Fig. 9.5), the ethanolamine conjugate of DHA (docosahexaenoic; 22:6n-3). Its concentration in animal tissues and human plasma increases when diets rich in fish or krill oil are consumed. Comparing a series of NAEs from n-3 and n-6 LC-PUFAs, we found DHEA to be the most potent anti-inflammatory compound in LPS-stimulated RAW264.7 macrophages [141]. Later studies suggested that anti-inflammatory effects of DHEA are at least partly independent from CB_1, CB_2 or PPARɣ receptors and probably take place via inhibition of eicosanoids produced through COX-2 [56]. Interestingly, DHEA was also reported to inhibit growth of prostate and breast cancer cell lines which was at least partly independent from CB_1 or CB_2 interaction [142, 143]. Similarly, DHEA was shown to stimulate neurite growth, synaptogenesis and glutamatergic synaptic activity in developing hippocampal neurons via (at least) cannabinoid receptor-independent mechanisms [144]. Another example is *N*-arachidonoyl dopamine (NADA). Like DHEA, NADA was found to be potent inhibitor of PGE2 synthesis in lipopolysaccharide (LPS) stimulated primary glia cells [145, 146].

4 Endocannabinoids and Targets in Disease

4.1 General Aspects, Targets and Examples

The broad involvement of the endocannabinoidome in various biological processes and its many connections with other systems in terms of ligands, receptors and

metabolic pathways explains why it is has been associated with so many disorders and diseases. However, it should be noted that associations with pathologies like those mentioned in Table 9.1 do not imply that suitable targets for prevention or intervention are at an easy reach. On the contrary, its wide abundance and high degree of pleiotropy present serious challenges to develop efficacious and specific drugs. It has also become clear that initial strategies to modulate the ECS have probably been too narrow and expectations too high. Two well-known examples in this respect are the experiences thus far with CB_1 inverse agonists (Sect. 4.2) and FAAH blockers (Sect. 4.3). Furthermore, it is also clear that changes in the expression of certain receptors or ligands are often the result of other (patho-) physiological processes instead of being part of a modifiable cause of a disease. As can be seen from the list of disease areas of interest (Table 9.1) many of these are of a chronic and multifactorial character. It is increasingly acknowledged that such disorders are often better managed by multiple target strategies, instead of a "one disease–one target" approach. This involves the use of promiscuous drugs or targeted drug (of drug–food) combinations [147]. These developments stimulated by the evolution of "omics" technologies, system biology and bioinformatics and the endocannabinoidome lends itself well for such an approach [148]. Table 9.1 lists a non-exhaustive overview of disease areas of interest. In the next sections, two of these are further elaborated viz weight management (Sect. 4.2) and inflammation (Sect. 4.3). For other field readers are referred to the literature.

4.2 The Endocannabinoidome in Weight Management

The modulation of food intake and energy metabolism is generally considered one of the most pivotal roles of the ECS. It has also been the most intensively studied topic in this field, in particular until 2008 when the withdrawal of rimonabant caused a dramatic change. The ECS modulates food intake and energy metabolism at different levels, starting from CB_1 receptors within the GI tract to the regulation of hedonic rewarding of foods in the brain [82–84, 190, 191]. From an evolutionary perspective it is thought that one of its main functions is as a pleiotropic regulator of energy uptake and storage and of non-homeostatic eating behaviour [192, 193]. In the past these mechanisms were biologically advantageous in order to survive periods of food shortage [194]. The discovery of the high abundance of CB_1 in brain, and the observation that CB_1 antagonists and reverse agonists induce a reduction of appetite and food-intake in animals fuelled an enormous activity of research in academia and industry, resulting in the market introduction of rimonabant 2006. Expectations, therapeutic and financial, were very high. The failure of rimonabant because of depression-related side-effects [13] shocked the research community and the pharmaceutical industry. By the end of 2008 at least nine companies terminated active development projects of CB_1 blockers. Next to rimonabant, which has been on the market in Europe but not in the USA, several related compounds were in advanced stages of development, including taranabant

Table 9.1 Non-exhausting overview of main disease areas in which the endocannabinoidome is of potential interest

Obesity and metabolic syndrome	See Sect. 4.2
Cardiovascular disorders	[149–154]
CNS disorders[a]	[155–161]
Neurodegenerative diseases (general)	[162, 163]
Alzheimer's disease	[164, 165]
Trauma/brain injuries	[166, 167]
Cancer	[5, 32, 168–171]
Intestinal diseases	[30, 172–178]
Inflammation[b]	Section 4.3
Pain[c]	[29, 136, 148, 179–182]
Skin diseases	[183–185]
Liver diseases	[186–189]

[a]Here used as a collective term for various disorders (psychosis, stress, anxiety, fear, addiction); only a few references are mentioned

[b]Inflammation in a general sense. Often there are links with (chronic) pain

[c]Here used as a collective term for different forms of pain (nociceptive, hyperalgesia, neuropathic pain)

(Merck), surinabant (Sanofi) and CP-945,598 (otanabant, Pfizer). In the meantime it has become clear that CB_1 receptors are also abundantly present outside the CNS [12, 191]. In fact, it is now assumed that the central effects of rimonabant are responsible for the short-term reduction of food-intake, whereas the more sustained effects on body weight and the improvement of insulin resistance and blood lipids are largely due to its peripheral actions. In the gut, CB receptors show a specific distribution, being largely distributed in the enteric nervous system (ENS) [178]. Both CB_1 and CB_2 receptors are found on enteric neurons, nerve fibres and nerve terminals in the ENS. The CB_1 receptor is found on nerve fibres throughout the wall of the gut, but with the highest density in the two ganglionated plexuses, the myenteric and submucosal plexus, of the ENS. CB_1 expression on Vagal afferents was found to be regulated by CCK [85] and high/low fat diets [86]. Stimulation of central CB_1 receptors, for example by anandamide has been shown to increase food-intake. Remarkably, stimulation of CB_1 receptors on Vagal afferents seems to do the opposite [87].

Notwithstanding the failure of rimonabant and other CB_1 blockers/inverse agonists, CB_1 receptors remain of interest as a pharmacological target. The presence of CB_1 receptors outside the CNS offers possibilities for treatment of type 2 diabetes and other complications of the metabolic syndrome. To improve tissue specific activity and reduce CNS side-effects so-called peripherally restricted CB_1 antagonists are under investigation [12, 28, 121, 191, 195]. Furthermore, the use of CB_1 neutral antagonists or partial agonists as opposed to inverse agonists such as rimonabant has been proposed as a strategy [191].

As described in Sect. 2.1 there exist also (at least) one natural weak CB_1 antagonist, THCV from *Cannabis* which might offer possibilities in this respect [17, 41, 42].

In addition to CB receptors, related receptors may offer interesting targets in weight management, including TRVP1 (Sect. 3.2.3) and GPR119 (Sect. 3.2.4).

Although not discussed in further detail are the possibilities to target the ECS in order to increase appetite or food-intake in general. The use of *Cannabis* preparations in AIDS and cancer patients for this purpose has already been introduced in Sect. 2.1. The ECS is also receiving interest in relation to eating disorders like anorexia and bulimia nervosa [161, 196, 197].

4.3 Inflammatory Processes

Several receptors which are modulated by endocannabinoids or their structural analogues are involved in the regulation of inflammation, pain and immune-functions in a broad sense [100]. Of particular interest are CB_2 (Sect. 3.2.2), TRVP1, TRPA1 and other TRP cation channels (Sect. 3.2.3), GPR18 (Sect. 3.2.6) and PPARs (Sect. 3.2.7), and this list is likely to increase. Furthermore, a number of endocannabinoids per se (Anandamide, 2-AG) and related compounds (PEA, SEA, OEA, DHEA, etc.) have shown anti-inflammatory and (or) immune modulating properties. Finally, the endocannabinoidome is deeply intertwined with other important lipid-based signalling systems including those regulated by COX and LOX. On the one hand, this broad involvement offers several potential targets for intervention. On the other hand, this complexity provides challenges in terms of specificity and side-effects. Some examples will be highlighted in this Section.

4.3.1 Modulators of Endocannabinoid Turnover

Inhibition of enzymes involved in the synthesis or breakdown of endocannabinoids, in particular DAGL, MAGL, FAAH or NAAA (*N*-acylethanolamine acid amidase) has been considered a manner to modulate inflammation and (or) pain. Diacylglycerol lipases (DAGLα and DAGLβ) are involved in the synthesis of 2-AG. Inhibition of DAGLβ has been found to lower 2-AG, as well as AA and eicosanoids, in mouse peritoneal macrophages in a manner that was distinct and complementary to disruption of cytosolic phospholipase-A2 [198]. Mono-acyl glycerol lipase (MAGL) catalyses the hydrolysis of 2-AG to arachidonic acids (AA). Inhibition of peripheral MAGL in rats using the selective MAGL inhibitor JZL184 was found suppressed LPS-induced circulating cytokines which in turn was suggested to modulate central cytokine expression [199]. In brain, AA formed by hydrolysis of 2-AG has been shown to serve as pool for pro-inflammatory eicosanoid synthesis, thus representing another crossroads between endocannabinoid and eicosanoid pathways [81]. MAGL-disrupted mice displayed neuroprotection in a model for Parkinson's disease but showed no haemorrhaging in the gut as seen with COX inhibitors [200]. Inhibition of Fatty Acid Amide Hydrolase (FAAH) aiming to increase fatty amide levels has also been considered as intervention strategy in

inflammation and (or) pain. A number of animal studies, for example with the inhibitor URB597, indeed showed reduction of inflammatory pain or modulation pro-inflammatory gene induction, although results were not always unambiguous [201, 202]. It has been suggested that inactivation of FAAH can modulate 2-AG tissue levels as well, either up or down, depending on the location [203]. Studies in human volunteers with FAAH inhibitors confirmed increased NAE levels, including that of AEA, OEA and PEA [204]. However, in a recent phase II clinical trial in patients with osteoarthritic knee pain the FAAH inhibitor PF-04457845 failed to show any effect [205, 206]. As FAAH activity was strongly inhibited and plasma NAE concentrations consistently elevated, it was suggested that alternative targets and pathways for breakdown might have counteracted the potentially beneficial effects of elevated anandamide levels on pain and inflammation [207]. Inhibition of NAAA provides an alternative approach to increase levels of for example PEA and OEA. Recently, the selective NAAA inhibitor ARN077 has been found to inhibit hyperalgesia and allodynia caused by inflammation or nerve damage [208]. Interestingly, the antinociceptive effects of ARN077 were prevented by the selective PPAR-α antagonist GW6471 and did not occur in PPAR-α knockout mice.

4.3.2 Endocannabinoid Congeners as Potential Anti-inflammatory Compounds

Several individual endocannabinoids, fatty amides and phytocannabinoids have been demonstrated to possess anti-inflammatory properties [100]. The n-3 LC-PUFA derived *N*-docosahexaenoylethanolamine (DHEA, Fig. 9.5) has already been described in Sect. 3.3. The same holds true for the *Cannabis*-derived compound CBD (Sect. 2.1). An interesting compound which is receiving increasingly attention is *N-Palmitoylethanolamide* (PEA, Fig. 9.5), an endogenous NAE originating from palmitic acid (C16:0), the most common saturated fatty acid found in animals [209]. Earliest reports on its anti-inflammatory properties date back to 1957. PEA shows a broad diversity of receptor affinities, including interactions with PPARα, GPR55 and TRVP1, as well as indirect activity via an "entourage" effect [137, 210]. The latter refers to a mechanism in which PEA reduces the enzymatic breakdown of AEA through competition for FAAH, resulting in higher AEA concentrations [211, 212]. The compound is presently receiving attention as potential drug or nutraceutical against chronic pain, (neuro-)inflammation and degenerative diseases of the central nervous system [137, 167, 209, 213, 214]. Increasing evidence indicates that non-neuronal cells within the CNS are crucially involved in mediating the effects of PEA [137, 215, 216]. These non-neuronal cells regulate inflammatory processes in the CNS and are key players in the communication between the immune system and the CNS during neurodegenerative disorders and in neuropathic pain. The C18 homologue of PEA, *N*-stearoyl ethanolamine (SEA) has also been associated with anti-inflammatory effects but this compound has been far less investigated [217].

5 Conclusions and Perspectives

More than two decades of research have changed our early view of the ECS. Initial expectations on the possibilities to develop new drug classes based on its key molecular targets have proven to be too high. It is now obvious that the "prototypical" ECS is deeply intertwined with other important signalling systems. Endocannabinoids have numerous bioactive congeners and metabolites, which often show "promiscuous" behaviour towards their receptors and other targets. This so-called endocannabinoidome is modulated by various endogenous (e.g. energy status, inflammation) and environmental factors in a time- and tissue-specific manner. The complexity and dynamics of the endocannabinoidome presents technical challenges and its understanding and modulation demands for a systems biology approach. At the same time the endocannabinoidome still holds many promises for both "food" and "pharmaceutical" applications as it is crucially involved in many disorders. Chronic diseases often involve tissue degeneration and remodelling, inflammation and pain, and are orchestrated by different interacting metabolic processes in which the "expanded" ECS is centrally involved.

Significant progress in their prevention and modulation is likely to come from a paradigm shift as it is currently taking place in the discovery and development process of drugs and nutritional products. These involve more subtle multiple-target strategies instead of a classical one disease–one target–one drug approach.

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