



The endocannabinoid system: an overview

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Upon the identification of anandamide (AEA) in the porcine brain, numerous studies contributed to the current state of knowledge regarding all elements that form the "endocannabinoid system (ECS)." How this complex system of receptors, ligands, and enzymes is integrated in helping to regulate fundamental processes at level of central nervous and peripheral systems and how its regulation and dysregulation might counteract disturbances of such functions, is nowadays still under investigation. However, the most recent advances on the physiological distribution and functional role of ECS allowed the progress of various research tools aimed at the therapeutic exploitation of endocannabinoid (eCB) signaling, as well as the development of novel drugs with pharmacological advantages. Here, we shall briefly overview the metabolic and signal transduction pathways of the main eCBs representatives, AEA, and 2-arachidonoylglycerol (2-AG), and we will discuss the therapeutic potential of new ECS-oriented drugs.

Keywords: anandamide, 2-arachidonoylglycerol, endocannabinoids, metabolic pathways, signal transduction

ENDOCANNABINOID SYSTEM: METABOLISM AND TARGETS OF ENDOCANNABINOID

Starting from 1992, when anandamide (AEA) was identified for the first time in the porcine brain (Devane et al., 1992), numerous studies contributed to the current state of knowledge regarding all elements that form the "endocannabinoid system (ECS)" (Maccarrone et al., 2010). Endocannabinoids (eCBs) are lipid mediators, isolated from brain and peripheral tissues that include amides, esters, and ethers of long chain polyunsaturated fatty acids; they mimic the action of Δ^9 -tetrahydrocannabinol (THC) in different biological processes. Until now, the most bioactive eCBs are anandamide (arachidonylethanolamide; AEA) and 2-arachidonoylglycerol (2-AG), yet the eCBs family includes also virodhamine, noladin ether, and *N*-arachidonoyldopamine (NADA), besides homo-linolenylethanolamide (HEA), docosatraenylethanolamide (DEA), and other cognate compounds such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (Figure 1).

eCBs are released "on demand" from membrane phospholipid precursors and, although AEA synthesis might be due to several metabolic routes (Muccioli, 2010), *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) is currently considered the major enzyme responsible for AEA production (Okamoto et al., 2009), whereas a specific phospholipase C followed by the activity of the *sn*-1-diacylglycerol lipase (DAGL) is responsible for 2-AG synthesis (Ueda et al., 2011). The cellular uptake from the extracellular to the intracellular space is ascribed to a purported "endocannabinoid membrane transporter (EMT)" that is likely to take up both AEA and 2-AG. However, while there is wide experimental evidence to support the concept that AEA transport across membranes is protein-mediated, conclusive evidence of its molecular identity is still lacking. Very recently, a partly truncated fatty

acid amide hydrolase-1 (FAAH-1) termed FAAH-1 like anandamide transporter (FLAT) has been reported in neural cells (Fu et al., 2011). After re-uptake, the biological activity of eCBs is ended by a FAAH, for AEA (McKinney and Cravatt, 2005), and/or by a specific monoacylglycerol lipase (MAGL), for 2-AG (Dinh et al., 2002). Additionally, other enzymes showing "amidase signature," such as FAAH-2 (Wei et al., 2006) and the *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (Tsuboi et al., 2005), which belongs to the cholesteryl esterase family, might bind with low affinity and hydrolyse AEA to release arachidonic acid and ethanolamine. Also cyclooxygenase-2 (COX-2), different lipoxygenase (LOX) isozymes and cytochrome P450 are able to accept AEA and 2-AG as a substrate, leading to the biosynthesis of prostaglandin-ethanolamides (Kozak et al., 2002) and -glyceryl esters (Kozak et al., 2001), hydroxy-anandamides, and hydroxyleicosatetraenoyl-glycerols (van der Stelt et al., 2002), respectively. For a comprehensive review on alternative pathways of eCBs see Rouzer and Marnett (Rouzer and Marnett, 2011). eCBs act principally through cannabinoid receptors, that include type-1 and type-2 (CB₁ and CB₂) receptors; more recently, it has been highlighted the ability of some CB and non-CB ligands to bind also to GPR55 (Glucksmann and Weich, 1999; Wise and Brown, 2001; Drmota et al., 2004; Pertwee, 2007; Ryberg et al., 2007; Lauckner et al., 2008), thus suggesting that the latter protein might act as a novel "type-3 (CB₃)" cannabinoid receptor (Moriconi et al., 2010). CB receptors are members of the large family of heptahelical G protein coupled receptors (GPCRs), activate Gi/o proteins (Pertwee et al., 2010). Anatomical studies have revealed that these receptors display a highly divergent pattern of distribution throughout the organism: CB₁ mainly present in the central nervous system (Herkenham et al., 1991) and, on the other hand, CB₂ mainly distributed in peripheral and immune cells (Munro et al., 1993). This topographical dichotomy has been

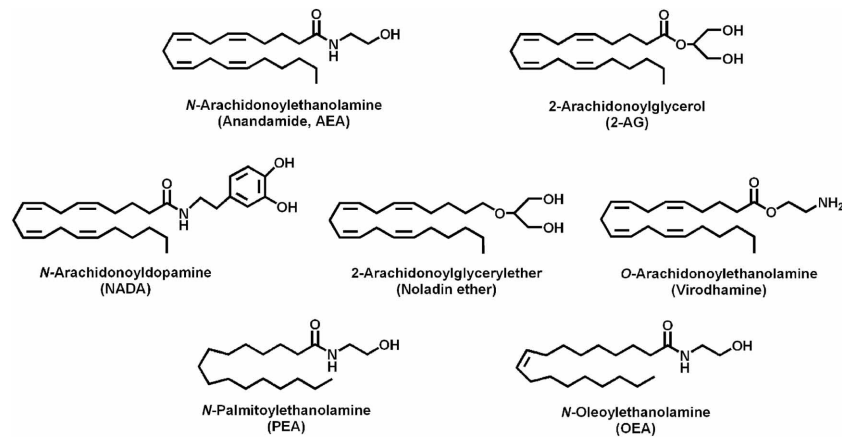


FIGURE 1 | Chemical structures of biologically active eCBs and of the eCB-like compounds.

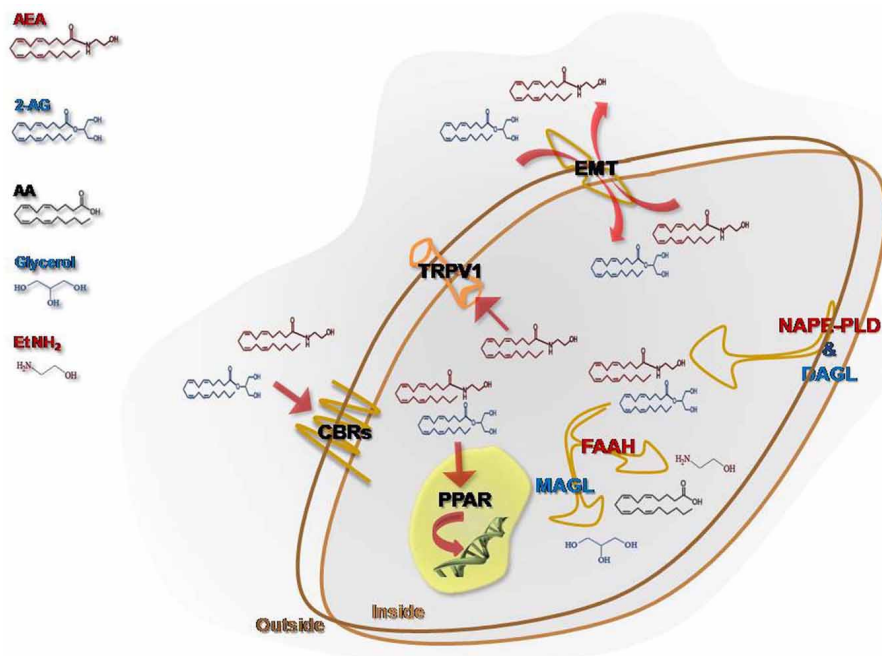


FIGURE 2 | Schematic representation of the main elements that constitute the endocannabinoid system. The synthesis of *N*-arachidonylethanolamine (AEA) is due to the activity of a NAPE-specific phospholipase D (NAPE-PLD), whereas a fatty acid amide hydrolase (FAAH) is responsible for its intracellular degradation to ethanolamine (EtNH₂) and arachidonic acid (AA). 2-Arachidonoylglycerol (2-AG) is released from membrane lipids through the activity of diacylglycerol lipase (DAGL), and it is

hydrolyzed by a cytosolic monoacylglycerol lipase (MAGL) that releases glycerol and AA. A purported endocannabinoid membrane transporter (EMT) clears AEA and 2-AG from the extracellular space, and takes them up into the cell. Both AEA and 2-AG trigger several signal transduction pathways by acting at their targets, CB₁, CB₂, GPR55, and nuclear PPARs. AEA, but not 2-AG, binds intracellularly also TRPV1, and thus it is also designated as a true endovanilloid.

revised by a number of studies documenting the presence of CB₁ in several non-neuronal cells and tissues (Gong et al., 2006), and of CB₂ in the brain stem (van Sickle et al., 2005) and in neuronal cells upon exogenous insults (Viscomi et al., 2009). In addition, the non-selective cationic channel type-1 vanilloid receptor (transient receptor potential vanilloid 1, TRPV1), usually activated by capsaicin and by noxious stimuli-like heat and protons (Di Marzo

and De Petrocellis, 2010), is an alternative target for AEA, but not for 2-AG. More recently, also nuclear receptors like the peroxisome proliferator-activated receptors (PPARs) have been added to the list of eCBs targets, activated under physiological and pathological conditions (Pistis and Melis, 2010). A schematic representation of eCBs, their receptors, biosynthetic and catabolic enzymes, as well as putative transporter, is depicted in **Figure 2**.

eCBS AND THEIR SIGNAL TRANSDUCTION PATHWAYS

The signal transduction pathways coupled to CB, TRPV1, and PPAR receptors are summarized in **Table 1**. Among the effects elicited by eCBs by binding to CB receptors, we should recall Ca^{2+} channels inhibition (including N-, P/Q-, and L-type channels), inhibition of adenylyl cyclase and subsequent decrease of cAMP-dependent protein kinase, which leads to decreased phosphorylation of the K^+ channels, regulation of ionic currents, activation of focal adhesion kinase, stimulation of mitogen-activated protein kinase (MAPK) cascades (Pertwee, 2006), and specifically ERK, p38 MAPK cascades (Derkinderen et al., 2001; Gertsch et al., 2004), and the stimulation of additional intracellular pathways including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway through CB_2 (Molina-Holgado et al., 2002).

Unlike CB_2 , CB_1 receptors are associated to special membrane microdomains, called “lipid rafts” (LR) that modulate CB_1 -dependent signaling pathways. The functional relationship between CB_1 and LR is affected by cholesterol content; in particular, membrane cholesterol enrichment in both primary and immortalized cell lines reduces the binding to CB_1 and subsequent G-protein dependent signaling through adenylyl cyclase and MAPK (Bari et al., 2005). Moreover, the disruption of LRs by cholesterol depletion modifies AEA-induced endocytosis of CB_1 , which apparently loses the capacity to be directed toward the lysosomal compartment. Therefore, LRs, besides representing a favorable platform to regulate CB_1 signaling, might also represent a cellular device for its intracellular trafficking (Sarnataro et al., 2005; Dainese et al., 2007). The general model to explain the neuromodulatory actions of AEA involves the release of eCBs from a postsynaptic neuron upon stimulation, then the back diffusion to presynaptic terminals, where AEA activates CB_1 receptors, thus modulating neuronal membrane permeability to Ca^{2+} e K^+ ions and the activity of adenylyl cyclase. The final outcome is a modified action of neurotransmitters (Di Marzo and De Petrocellis, 2010).

Table 1 | Signal transduction pathways triggered by eCBs at different target receptors.

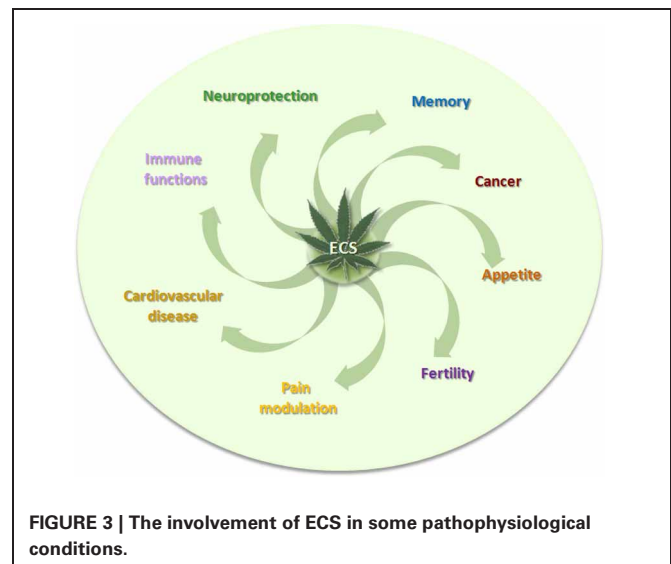
Receptor	Effect
CB_1 and CB_2	<ul style="list-style-type: none"> ↓ Adenylyl cyclase ↑ Focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK) ↑ ERK, p38 through CB_1, and PI3K/Akt through CB_2 ↑ K^+ channels ↓ Ca^{2+} channels
GPR55	<ul style="list-style-type: none"> ↑ Intracellular $[Ca^{2+}]$ ↑ RhoA, Rac, and Cdc42 ↑ ERK phosphorylation
TRPV1	<ul style="list-style-type: none"> ↑ Intracellular $[Ca^{2+}]$ ↑ Caspases ↑ Cytochrome c release ↑ Mitochondrial uncoupling ↑ Pro-apoptotic kinases
PPARs	<ul style="list-style-type: none"> ↑ ROS ↑ Tyrosine kinases ↑ Adiponectin and lipoprotein lipase

The activation of GPR55, the purported “ CB_3 ” cannabinoid receptor, has been linked to (1) intracellular Ca^{2+} increase (Lauckner et al., 2008); (2) activation of the small GTPase proteins RhoA, Rac, and Cdc42 (Ryberg et al., 2007; Henstridge et al., 2009), and (3) ERK phosphorylation (Oka et al., 2007, 2009). Additionally, by triggering PPARs, eCBs exert a variety of long-term effects *via* genomic mechanisms and rapid non-genomic actions, which are opposite to those evoked by activation of “classical” surface cannabinoid receptors (Pistis and Melis, 2010). As a consequence, PPARs activation affects several physiological and pathological processes, such as lipid metabolism, energy balance, and feeding behavior, neuroprotection, epilepsy, circadian rhythms, inflammation, addiction, and cognitive functions (Pistis and Melis, 2010). However, AEA can also act as a modulator of other signaling pathways and, in fact, it has been observed that muscarinic and glutamate receptors have allosteric sites for AEA binding (Lanzafame et al., 2004). In this context, it should be underlined that there are several findings showing that eCBs modulate the signaling of several neuropeptides and hormones (Manzanares et al., 1999; Beinfeld and Connolly, 2001; Ghozland et al., 2002). This highly complex network of interactions is reflected in the multifaceted modulatory effects of eCBs on the regulation of brain and behavioral functions (López-Moreno et al., 2008).

PHYSIOLOGICAL ACTIONS OF ECS AND THERAPEUTIC PERSPECTIVES

The presence of ECS in vertebrates, mammals, and humans implies a role in several physiological processes, including appetite, cancer, cardiovascular diseases, fertility, immune functions, memory, neuroprotection, and pain modulation (Ligresti et al., 2009; Maccarrone et al., 2010) (**Figure 3**).

In the last 10 years, it has become clear that a dysregulation of ECS is connected to pathological conditions, and thus its modulation through inhibition of metabolic pathways and/or agonism or antagonism of its receptors has an enormous potential for research and intervention in multiple areas of human health.



Therefore, based on the therapeutic potential of THC, known since centuries as medicine for its palliative effects in several pathologies, plant-derived cannabinoids, synthetic cannabinoids, and eCBs have been tested as novel therapeutics in a wide range of clinical trials.

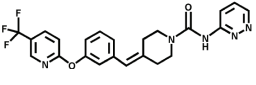
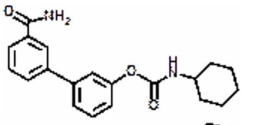
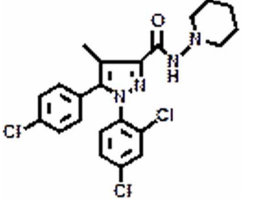
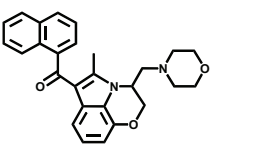
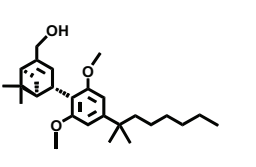
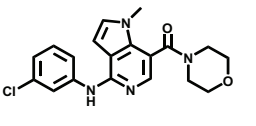
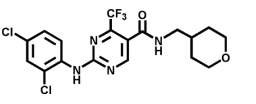
The neuroprotective effect of eCBs might be mediated by either CB₁- or CB₂-dependent mechanisms. Research studies using *cb1^{-/-}* knock-out mice showed an increased mortality rate and an increased infarct area in cerebral ischemia models (Parmentier-Batteur et al., 2002). It has been reported that the administration of the CB₁ synthetic agonist WIN 55.212–2 attenuated the neurological damage and reduced infarct size in artery occlusion induced in rats (Nagayama et al., 1999), and additionally it reduced the glial damage after hypoxic-ischemic brain injury in preterm lambs (Alonso-Alconada et al., 2010). The presence of CB₂-positive cells in the brain during injury and in inflammatory neurodegenerative disorders might provide a novel strategy for cannabinoid-mediated intervention against stroke-induced neurodegeneration, without the unwanted psychoactive effects of CB₁ receptor stimulation (Cunha et al., 2011). O-3853

and O-1966, two selective CB₂ agonists, administered 1 h before transient middle cerebral artery occlusion, significantly decreased the mobilization of white blood cells and their adherence to vascular endothelial cells, reduced the infarct size, and improved motor function after transient focal ischemia (Zhang et al., 2007, 2009).

According to these observations, pain management is preferably handled using CB₂ agonists, such as HU-308 and AM-1241, which display significant relief in inflammatory and neuropathic pain models, without exhibiting central nervous system side effects (Hanus et al., 1999; Yao et al., 2006). In this context, new selective CB₂ receptor modulators, designed by Glaxo Smith Kline as derivatives of pyrimidinecarboxamide, have been tested as good clinical candidates to treat inflammatory, acute, and chronic pain (Giblin et al., 2007, 2009).

In the past, several reports documented that the selective pharmacologic antagonism of the CB₁ receptor improves lipid abnormalities associated with obesity, as well as neurodegenerative diseases and nicotine or alcohol dependence (Centonze et al., 2007; Di Marzo, 2008). Following the good outcome obtained in

Table 2 | Chemical structures and therapeutic potential of some ECS-targeted molecules.

Chemical structure	Compound	ECS target	Diseases	References
	PF-04457845	FAAH	Pain, Osteoarthritis	Ahn et al., 2011
	URB 597		Anxiety, Cannabis dependence, Hyperalgesia	Bortolato et al., 2007
	SR141716A	CB ₁	Eating disorder	Christopoulou and Kiortsis, 2011
	WIN 55.212–2		Ischemic stroke, Brain injury	Nagayama et al., 1999; Alonso-Alconada et al., 2010
	HU-308	CB ₂	Neuropathic pain	Hanus et al., 1999
	GSK554418A		Acute/chronic pain	Giblin et al., 2009
	GW842166X		Inflammatory pain	Giblin et al., 2007

various clinical trials, the best known CB₁ blocker SR141617A, also called rimonabant (and commercially known as Acomplia®) was released on the worldwide market as anti-obesity drug, but only few months later it was withdrawn because of increased rates of depression, anxiety, and suicide among patients who received it (Christopoulou and Kiortsis, 2011). In addition, further concerns were raised considering the possible side effects of this weight loss pill on the reproductive functions and human infertility (Bari et al., 2011).

Alternative strategies to treat pain syndromes, such as neuropathic pain, fibromyalgia, but also spontaneous abortion, headache, psychiatric disorders, and neurodegenerative diseases, are based on the enhancement of the eCB tone, through the inhibition of eCBs-hydrolyzing enzymes (Lichtman and Chapman, 2001). The most promising FAAH inhibitor seems to be URB597 (also named KDS-4103), which has biochemical and behavioral effects during both sub-acute and chronic treatments. In rodents, once-daily dosing of URB597 for five weeks elicits antidepressant effects in chronically stressed animals, without altering CB₁ receptor mRNA levels (Bortolato et al., 2007). Pfizer and Vernalis pharmaceutical companies focused on FAAH as main target to design and develop new molecules (PF-04457845 and V158866, respectively), that are being tested in clinical studies as potential therapies for a range of pain disorders, including osteoarthritis

(Ahn et al., 2011). It is noteworthy that FAAH inhibitors, because of their own pharmacological properties, are attractive remedial also for cannabis dependence; in fact, they do not appear to evoke tolerance following long-term administration, and they do not display significant abuse liability (Clapper et al., 2009). **Table 2** reports some agonists, antagonists, and/or inhibitors of ECS designed for the treatment of several pathological conditions.

CONCLUSION

Almost 20 years after the identification of AEA, all members of ECS are nowadays considered intriguing targets for the development of selective and specific compounds able to modulate human pathophysiology. A deeper and more detailed understanding of proteins involved in eCBs metabolism and signal-transduction pathways could help to design compounds that might prolong the activity of eCBs in a time- and site-dependent way, excluding undesired psychotropic effects, and to develop transgenic mice, where different ECS elements can be knocked down or knocked in, allowing innovative therapeutic strategies in a vast panorama of pathologies.

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