Pharmacological Research 60 (2009) 77–84

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Review

The endocannabinoid system: Its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation

Vincenzo Di Marzo*

Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Comprensorio Olivetti, 80078 Pozzuoli, Naples, Italy

ARTICLE INFO

Article history:
Received 28 January 2009
Accepted 19 February 2009

Keywords:
Cannabinoid
Endocannabinoid
Vanilloid
Inhibitors
Homeostasis
CB_1
CB_2
TRPV1
Anandamide
2-Arachidonoylglycerol
FAAH
MAGL
DAGL

ABSTRACT

The endocannabinoid signalling system includes: (1) at least two G-protein-coupled receptors, known as the cannabinoid CB_1 and CB_2 receptors and discovered following studies on the mechanism of action of Δ^9-tetrahydrocannabinol, the major psychoactive principle of the hemp plant *Cannabis sativa*; (2) the endogenous agonists at these receptors, known as endocannabinoids, of which anandamide and 2-arachidonoylglycerol are the best known; and (3) proteins and enzymes for the regulation of endocannabinoid levels and action at receptors. The endocannabinoid system is quite widespread in mammalian tissues and cells and appears to play a pro-homeostatic role by being activated following transient or chronic perturbation of homeostasis, and by regulating in a local way the levels and action of other chemical signals. Compounds that selectively manipulate the action and levels of endocannabinoids at their targets have been and are being developed, and represent templates for potential new therapeutic drugs.

Contents

1. The endocannabinoid system, its components and their regulation

The discovery of the major psychotropic component of the preparations from *Cannabis sativa*, the lipophilic compound Δ^9-tetrahydrocannabinol (THC) [1], was not immediately followed by the molecular characterization of the corresponding receptor in the mammalian brain. More than two decades had to be waited until the first THC-specific receptor, named cannabinoid receptor type-1 (CB_1), could be first identified [2] and then cloned after the screening of several previously characterized orphan G-protein-coupled receptors (GPCRs) for their affinity for THC [3]. The second cannabinoid receptor, named CB_2, identified by means of homology cloning, turned out to be rather different from CB_1 both in its amino acid sequence and its localization in mammalian tissues [4]. Whilst CB_1 was shown to be extremely abundant in the brain, and hence suggested to be responsible for THC psychoactivity, CB_2 was expressed in its highest levels in immune cells. The cloning of the cannabinoid receptors opened the way to the identification of their endogenous ligands, or endocannabinoids. The first endocannabinoid to be discovered was anandamide (*N*-arachidonoyl-ethanolamine) [5], a finding soon to be followed by the observation that an already known endogenous metabolite, 2-arachidonoyl-glycerol (2-AG), also exhibits high affinity for CB_1 and CB_2 receptors [6,7]. Other

* Tel.: +39 081 8675093; fax: +39 081 8041770.
E-mail address: vdimarzo@icmib.na.cnr.it.

1043-6618/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2009.02.010

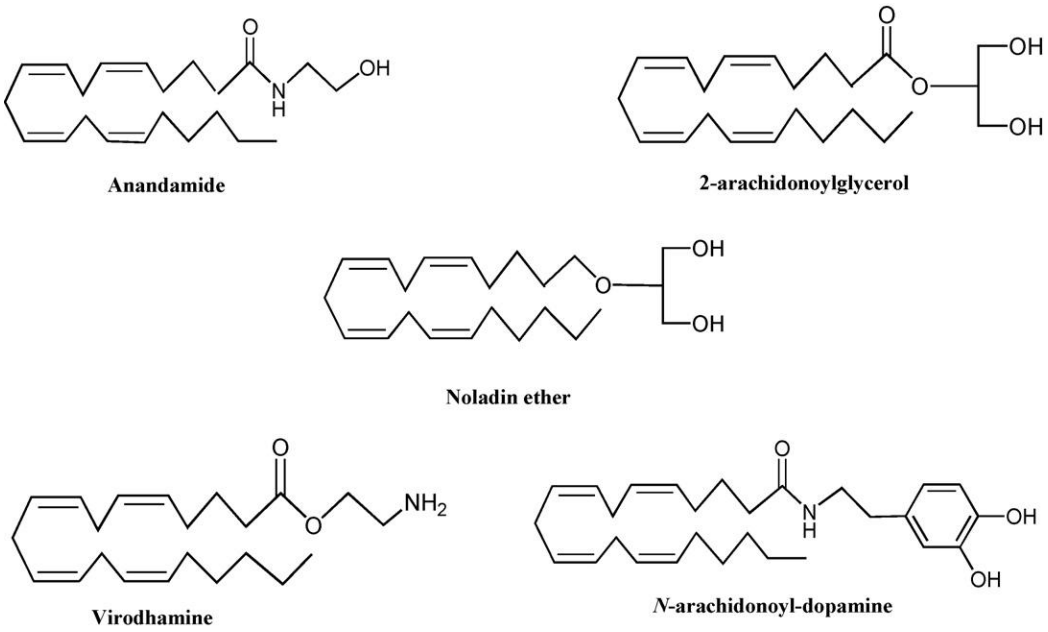

Fig. 1. Chemical structures of the proposed endocannabinoids.

endocannabinoids (Fig. 1) have also been proposed during the last 10 years, including 2-arachidonyl-glycerol ether (noladin ether) [8], *N*-arachidonoyl-dopamine (NADA) [9,10] and virodhamine [11], but their pharmacological activity and metabolism has not yet been thoroughly investigated. Therefore, anandamide and 2-AG are still referred to as the "major" endocannabinoids. More recently, the first potent endogenous antagonist/inverse agonist of CB_1 receptors was also identified. This is a nonapeptide known as hemopressin, isolated by various tissues including the brain [12], and previously found to induce hypotensive effects that would not be entirely in agreement with the similar activity described for CB_1 agonists. Further studies on the pharmacology and regulation of the levels of this peptide during physio-pathological conditions are required in order to substantiate its role as endogenous CB_1 blocker.

The catabolic pathways and enzymes (Table 1) for anandamide and 2-AG have been largely investigated and partly identified. *N*-Arachidonoyl-phosphatidylethanolamine (NArPE) and diacylglycerols (DAGs) with arachidonic acid on the 2-position act as the major biosynthetic precursors of anandamide [13] and 2-AG [14–16], respectively. NArPE is produced from the transfer of arachidonic acid from the *sn*-1 position of phospholipids to the nitrogen atom of phosphatidylethanolamine [17], whereas DAG precursors for 2-AG derive mostly from the phospholipase C-catalysed hydrolysis of phosphatidylinositol [16] and, in certain cells, from the hydrolysis of phosphatidic acid [18]. The two endocannabinoids are inactivated essentially by enzymatic hydrolysis of their amide and ester bonds, and the major enzymes responsible for these reactions have been cloned from several mammalian species and are known as fatty acid amide hydrolase (FAAH) [19] and monoacylglycerol lipase (MAGL) [20,21], for anandamide and 2-AG, respectively. Biosynthetic enzymes for endocannabinoids have been also cloned. Two *sn*-1-selective DAG lipases, named DAGL-α and DAGL-β, are responsible for 2-AG biosynthesis in cells and tissues [22], whereas the enzyme catalysing the direct conversion of NArPE into anandamide is known as *N*-acylphosphatidyl-ethanolamine-specific phospholipase D (NAPE-PLD) [23]. Finally, a specific process through which endocannabinoids, according to the direction of their gradient of concentrations across the plasma membrane, are either taken up by cells following cannabinoid receptor activation, or released from cells following endocannabinoid biosynthesis, has been proposed by some authors [13,24–26], but not others [27,28]. This mechanism appears to be pharmacologically distinct from FAAH or MAGL [29,30] or CB_1 receptors [31], although it not yet been identified from a molecular point of view.

Several alternative enzymes for the biosynthesis of anandamide from NArPE, and for the inactivation of 2-AG to glycerol and arachidonic acid, have been recently proposed (Table 1). Since NAPE-PLD "knock-out" mice do not exhibit reduced levels of anandamide in most tissues [32], this endocannabinoid was suggested to be formed also from the sequential cleavage of the two *sn*-1 and 2-acyl groups of NArPE, catalysed by alpha/beta-hydrolase 4, followed by the phosphodiesterase-mediated hydrolysis of glycerophospho-anandamide [33]. The formation of phospho-anandamide from the hydrolysis of NArPE catalysed by phospholipase C enzyme(s), followed by its conversion into anandamide by protein tyrosine phosphatase N22, is another possible biosynthetic route [34,35]. Finally, the biosynthesis of anandamide might also occur via conversion of NArPE into 2-lyso-NArPE by a soluble form of phospholipase A_2, followed by the action of a lysophospholipase D [36].

MAGL seems to be only one of several hydrolases that may catalyse 2-AG hydrolysis [37]. FAAH seems to control this reaction under certain conditions [38], whereas alpha/beta-hydrolases 6 and 12 were also found to recognize 2-AG as substrate. In whole brain homogenates, however, MAGL is the major contributor to 2-AG inactivation, although the situation in vivo might be different. Studies with specific inhibitors of these enzymes (see below) as well as with the corresponding "knock-out" mice are required to provide an answer as to what enzyme, and when and where, is most responsible for 2-AG hydrolysis.

Studies carried out using FAAH null mice revealed another potential pathway also for anandamide catabolism, different from enzymatic hydrolysis [39]. In fact, the accumulation of *N*-acylethanolamines in these transgenic mice allowed to identify the presence of *O*-phosphorylcholine-derivatives of these compounds, which do not appear to be good substrates for FAAH and are hydrolysed back to the parent compounds by the choline-specific phosphodiesterase NPP6. It is not clear how *O*-phosphorylcholine-*N*-acyl-ethanolamines are formed, and this pathway might represent either a way of storing and then releasing anandamide and its congeners or a new mechanism to inactivate them.

Table 1
"Old" and "New" enzymes for anandamide and 2-arachidonoylglycerol (2-AG) biosynthesis (Bio) and degradation (Deg).

Enzyme	Anandamide		2-AG	
	Bio	Deg	Bio	Deg
N-Acylphosphatidylethanolamine-selective phospholipase D (NAPE-PLD) 393 residues. Belongs to the metallo-beta-lactamase superfamily. Hydrolyses *N*-acyl-phosphatidylethanolamines (NAPEs) to produce *N*-acylethanolamines (NAEs) and phosphatidic acid. Binds 1 or 2 zinc ions per subunit, activity is stimulated by divalent cations including Ca^{2+}. Localized to intracellular membranes.	X			
Fatty acid amide hydrolase (FAAH) 579 residues. Belongs to the amidase family. It degrades bioactive fatty acid amides like oleamide, the endogenous cannabinoid, anandamide and myristic amide to their corresponding acids. Hydrolyses polyunsaturated and monounsaturated substrates preferentially as compared to saturated substrates. It is a homodimer that seems to be attached to intracellular membranes and to a portion of the cytoskeletal network. It is highly expressed in the liver, brain, small intestine, pancreas, skeletal muscle and testis. Also expressed in the kidney, lung, placenta and prostate. It might also catalyse the condensation between fatty acids and amines.		XX		X
Diacylglycerol lipase (DAGL) α 1042 residues. Belongs to the AB hydrolase superfamily and is a *sn*-1-specific diacylglycerol lipase. Catalyses the hydrolysis of diacylglycerol (DAG) to 2-AG. It is localized in the plasma membrane, possibly by means of four transmembrane domains, and is stimulated by Ca^{2+}. Highly expressed in brain and pancreas. Its isoform (DAGLβ) has a smaller size.			XX	
Monoacylglycerol lipase (MAGL) 303 residues (human). Belongs to the AB hydrolase superfamily. Prefers monoacylglycerols with at least one double bond. The deduced 303-amino acid protein shares 84% identity with mouse MAGL. Is expressed in wide variety of tissues. Mouse and human MAGL both have an N-terminal his-gly dipeptide, a characteristic of lipases, and a catalytic triad of ser122, asp239, and his269.				XXX
α,β-Hydrolase domain containing 4 (ABHD-4) 342 residues. Belongs to peptidase S33 family. Lysophospholipase selective for *N*-acyl phosphatidylethanolamine (NAPE). Hydrolyses substrates bearing saturated, monounsaturated, polyunsaturated *N*-acyl chains. Shows no significant activity towards other lysophospholipids, including lysophosphatidylcholine, lysophosphatidylethanolamine and lysophosphatidylserine. Thr-291 is present instead of the conserved His which is expected to be an active site residue.	X			
α,β-Hydrolase domain containing 6 (ABHD-6) 337 residues. Belongs to the AB hydrolase superfamily. Signal-anchor transmembrane.				X
α,β-Hydrolase domain containing 12 (ABHD-12) Belongs to the serine esterase family. Two isoforms produced by alternative splicing; the isoform 1 has been chosen as the 'canonical' sequence, whilst isoform 2 differs from the canonical sequence of 387–398 residues.				X
Tyrosine-protein phosphatase non-receptor type 22 (PTPN22) 807 residues. Belongs to the protein-tyrosine phosphatase family and catalyses the dephosphorylation of phosphotyrosine peptides. Cytoplasmatic enzyme predominantly expressed in lymphoid tissues and cells. Isoform 1 is expressed in thymocytes and both mature B and T-cells.	X			

Enzymes of the arachidonate cascade, i.e. cycloxygenase-2 (COX-2) and lipoxygenases, as well as cytochrome p450 enzymes, might intervene in alternative pathways for endocannabinoid inactivation [40]. The cycloxygenase-2 catalysed oxidation of anandamide, followed by the action of various types of prostaglandin synthases, might afford prostaglandin-ethanolamides (also known as "prostamides") [41], which are resistant to hydrolysis [42], and, at least in the case of prostamide $F_{2\alpha}$, activate a heterodimer between the FP receptor for prostaglandin $F_{2\alpha}$ and a splicing variant of such receptor [43]. Likewise, COX-2-catalysed metabolism of 2-AG might lead to prostaglandin glycerol esters (or glyceryl-prostaglandins) [44,45], one of which, glycerylprostaglandin E_2, activates an as yet unidentified GPCR at very low concentrations [46], and is not hydrolysed by FAAH or MAGL [47]. Oxygenation of 2-AG and/or anandamide might also occur *in vitro* via 12- and 15-lipoxygenases to the corresponding hydroperoxy- and hydroxy-derivatives [48–50], or by cytochrome p450 oxygenases to epoxyeicosatetraenoyl-anandamides [51,52]. The metabolites obtained in this case are usually still active at cannabinoid receptors. Therefore the biological relevance of these reactions remains to be established, and so does the actual presence of oxygenation products of endocannabinoids in living animals.

Both cannabinoid CB_1 and CB_2 receptors are mostly coupled to $G_{i/o}$ proteins, through the α subunits of which they inhibit adenylate cyclase and stimulate mitogen-activated protein kinases (MAPK) [53]. Typical $G_{i/o}$-mediated intracellular events coupled only to CB_1 activation are the inhibition of voltage-gated calcium channels (VGCCs) of most types, including P/Q, N and L-type channels, and the stimulation of inwardly rectifying K^+ channels [53–55]. Furthermore, increasing evidence exists for the capability, in certain cell types, of CB_1 receptor agonists, including endocannabinoids like 2-AG and anandamide, to directly stimulate: (1) the hydrolysis of PIP_2 by PLC-β, with subsequent release of inositol-1,4,5-phosphate (IP_3) and Ca^{2+} mobilization from the ER via either $G_{q/11}$-mediated or $G_{i/o}$-mediated mechanisms [56–59]; and (2) the modulation of the phosphoinositide-3-kinase (PI3K)-mediated signalling cascade via $G_{i/o}$ – described to be of either positive or negative nature depending on the cell type [60–64] – thereby affecting the downstream Akt/protein kinase B pathway. Other intracellular signalling effects described for both CB_1 and CB_2 receptors are the release of nitric oxide (NO) [65–67] and the subsequent activation of cGMP levels [68,69], whereas CB_2 is coupled also to increased release of ceramide [53].

Probably the best established non-CB_1 non-CB_2 receptor for anandamide and NADA, but not 2-AG, is the transient receptor potential vanilloid type-1 (TRPV1) receptor, a non-selective cation channel belonging to the large family of the transient receptor potential (TRP) channels, and activated by noxious heat (>42 °C), low pH (<6.0) and the hot chilli pepper active constituent, capsaicin [70,71]. An increasing number of experimental data, in some cases employing also TRPV1 null mice [72], suggests that this protein mediates some of the pharmacological effects of anandamide [71,38]. Evidence obtained *in vitro* also suggests that anandamide antagonizes another TRP channel, the TRP of melastatin type-8 (TRPM8), which is responsible for the cooling sensation induced by menthol and <25 °C temperatures [73,74].

Recent evidence, again limited to *in vitro* experiments, suggests that some plant and synthetic cannabinoids as well as endocannabinoids might bind to the orphan GPCR, GPR55 [75–77]. This is a protein present in several tissues and organs, including the brain,

and showing <20% sequence homology with CB_1 and CB_2. Unfortunately, the few papers published on this issue have often reported conflicting data with regard to either the potency or efficacy of endocannabinoids as GPR55 agonists, whereas other studies [78,79] did not even confirm the capability of either anandamide or 2-AG to exert this effect. GPR55 "knock-out" mice are available and their use is recommended to establish whether or not some of the *in vivo* actions of endocannabinoids are reduced or absent in these transgenic animals.

2. Anatomy of the endocannabinoid system, its general strategy of action and its pathological disruption

We now know that both CB_1 and CB_2 receptors are much more widely distributed than originally believed. For example, the liver is now established as a source of low, but nevertheless functionally important, amounts of CB_1 [80]. CB_2 receptors, the existence of which in the brain had been initially ruled out, were shown to be expressed in low amounts also in this organ and not only during neuroinflammatory conditions [81–83]. As a consequence, the original idea that CB_1 receptors played a role almost uniquely in the brain, and CB_2 in the immune system, has evolved into the concept that both cannabinoid receptor types can control both central and peripheral functions, including neuronal development, transmission and inflammation, cardiovascular, respiratory and reproductive functions, hormone release and action, bone formation and energy metabolism, as well as cellular functions, such as cell architecture, proliferation, motility, adhesion and apoptosis [84–87]. Accordingly, not only the expression level of cannabinoid receptors, but also the tissue concentrations of the "major" endocannabinoids undergo significant changes following physiological and pathological stimuli [88–90]. This "plasticity" of the endocannabinoid system is clearly observed in the CNS, where it underlies adaptive, pro-homeostatic responses to chronic stress, neuronal excitotoxicity and damage, and neuroinflammation [91], as well as more physiological mechanisms such as synaptic strength in cognitive, motivational and affective processes and their pathological alterations [92]. The biosynthesis, action and degradation of endocannabinoids are triggered "on demand" and are normally restricted in time and space, also thanks to lipophilic nature of these compounds, their phospholipid-dependent biosynthetic pathways and the Ca^{2+}-sensitivity of some of their biosynthetic enzymes. This allows for the pro-homeostatic action of CB_1 and/or CB_2 activation, which usually exerts a general "protective" function.

Also the anatomical distribution of the metabolic enzymes and receptors of the endocannabinoids support their proposed pro-homeostatic strategy of action. In the brain, for example, the biosynthetic and degradative enzymes for 2-AG are localized, with respect to CB_1 receptors, in a way that post-synaptic neurons, which express the DAGL-α in dendritic spines and somatodendritic compartments, by producing and releasing this endocannabinoid, can control the activity of the complementary pre-synaptic neurons, where the CB_1 receptor is often expressed [93]. This "retrograde" modulatory action is terminated by MAGL expressed on the same pre-synaptic terminal. CB_1 activation, then, by reducing the activity of voltage-activated Ca^{2+} channels and enhancing that of inwardly rectifying K^+ channels, can inhibit the release of neurotransmitters [53,94]. This paracrine signalling mechanism represents a "circuit-breaking" mechanism [93] and, hence, can re-establish an excessive activity of the post-synaptic neurons, such as during certain pathological neurological conditions [91–93]. In the female reproductive system, paracrine effects of endocannabinoid concentration gradients in the oviduct and uterus control the exact site of embryo implantation [95].

Another example of endocannabinoid-mediated paracrine mechanism has been recently described to occur in the liver, and suggested, instead, to participate in a pathological condition, rather than counteract it. In fact, Jeong and colleagues [96] found that chronic ethanol feeding increases the hepatic expression of CB_1 receptors and upregulates the levels of 2-AG and of its biosynthetic enzyme DAGLβ selectively in hepatic stellate cells. Co-culture of wild-type, but not that of CB_1 receptor-deficient, hepatocytes with stellate cells from ethanol-fed mice resulted in the upregulation of CB_1 receptors and lipogenic gene expression. The authors concluded that paracrine activation of hepatic CB_1 receptors by stellate cell-derived 2-AG mediates ethanol-induced steatosis through increasing lipogenesis and decreasing fatty acid oxidation [96]. Indeed, the tight time- and space-selectivity of endocannabinoid action might be lost during chronic conditions, in which endocannabinoids might start acting for a longer time, or at receptors located in cells that they were not initially supposed to target, thus contributing to the symptoms and progress of degenerative disorders. This might explain why, often for the same type of pathological conditions, not only "enhancers" of endocannabinoid action (such as FAAH and MAGL inhibitors), but also cannabinoid receptor antagonists might exert beneficial actions [88] (see below).

The general strategy of action of anandamide in the brain might be more complex than that of 2-AG due to the following observations: (1) unlike MAGL, FAAH is mostly located post-synaptically and in intracellular membranes, and this might not allow for a rapid inactivation of anandamide action at pre-synaptic neurons [97]; (2) unlike DAGL-α, NAPE-PLD is often (but not always) located pre-synaptically and in intracellular membranes [98–100] (although this protein is clearly not the only biosynthetic enzyme for anandamide); (3) anandamide also activates TRPV1, which is can be coupled to glutamate release in the brain, when expressed pre-synaptically, or to inhibition of DAGL-α, and therefore of 2-AG levels and retrograde signalling activity at CB_1, when expressed post-synaptically [101–105]. These data indicate for intracellular anandamide a potential role as a mediator acting at TRPV1 on a cytosolic binding site, and controlling Ca^{2+} homeostasis [106] and/or 2-AG biosynthesis [105], and for extracellular anandamide a potential "anterograde" activity at the post-synaptic targets of this compound [98].

3. Tools for the study of endocannabinoid biology as new leads for drug development

Several pharmacological tools for the study of the endocannabinoid system have been developed, and comprehensive reviews of the properties of those tools that have been most widely used were recently published [88,107]. These tools can be grouped functionally into five super-families, i.e.: (i) "indirect" cannabinoid receptor agonists (i.e. inhibitors of endocannabinoid inactivation), (ii) "direct" cannabinoid receptor agonists, (iii) "indirect" antagonists of cannabinoid receptors (i.e. inhibitors of endocannabinoid biosynthesis), (iv) cannabinoid receptor inverse agonists and antagonists, and (v) cannabinoid receptor allosteric modulators. Each of these super-families can be divided into various families of compounds, for a total of twelve such families:

(1) Inhibitors of endocannabinoid cellular uptake. The most widely used members of this category are AM404, LY-2183240, VDM11, UCM707, OMDM-1 and -2 and AM1172, in increasing order of selectivity. Recently, more potent and/or selective uptake inhibitors have been developed, including potentially covalent inhibitors [108], and compounds that have proved to be very potent also in vivo in an animal model of spasticity, the most potent of which was O-2093 [109]. Furthermore, the in vivo pharmacology of some tetrazole uptake inhibitors [110] was shown to be clearly different from that of

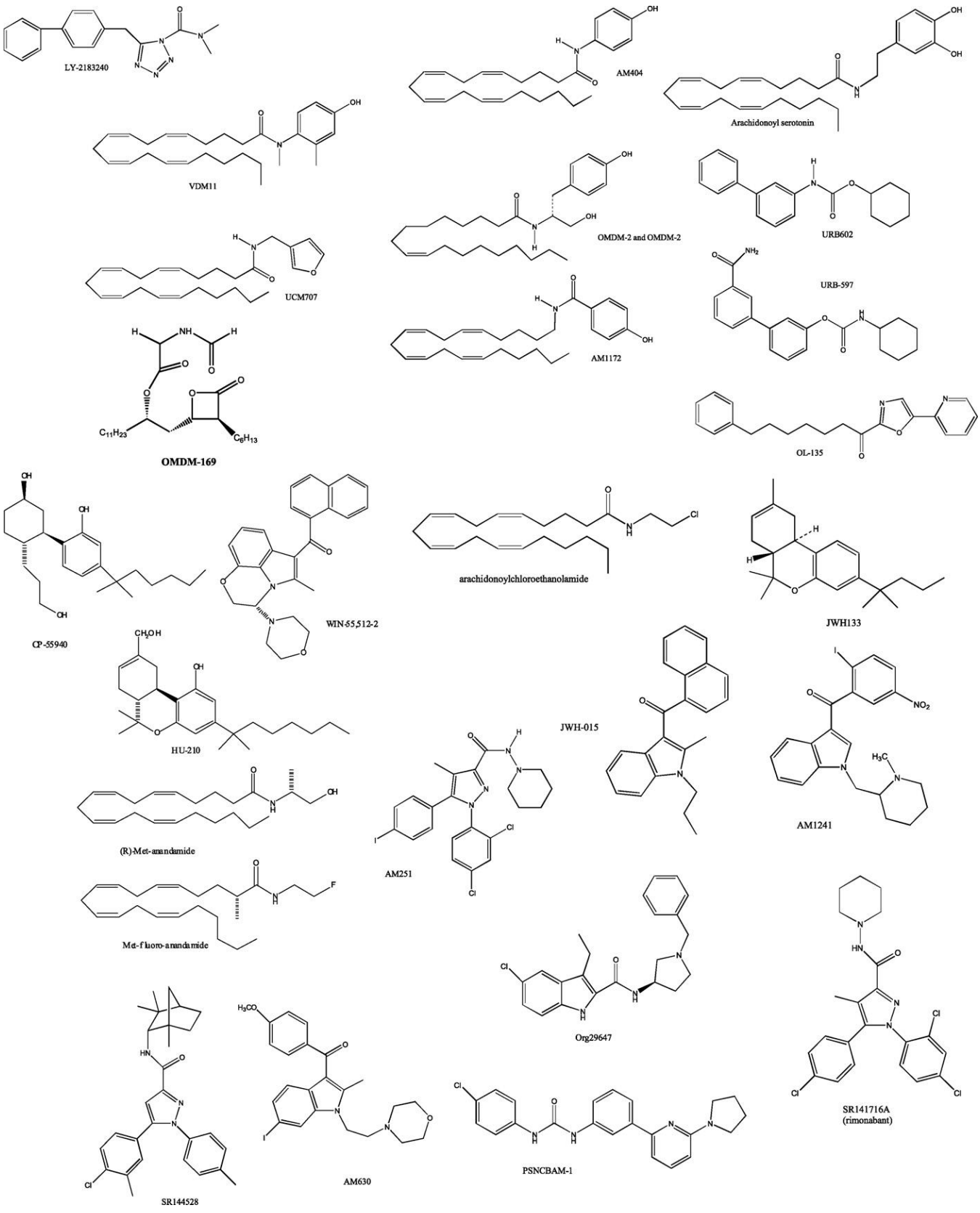

Fig. 2. Chemical structures of some of the pharmacological tools used to investigate the endocannabinoid system.

structurally similar FAAH inhibitors [29]. The potential therapeutic applications of these compounds include: neuropathic and inflammatory pain, post-traumatic stress disorders, anxiety, depression, Parkinson's and Alzheimer's disease, motor disturbances in multiple sclerosis, cancer cell proliferation, inflammatory bowel disorders, hypertension, high intraocular pressure and glaucoma, emesis and insomnia [88]. However, the further development of these inhibitors is hindered by the fact that the mechanism underlying endocannabinoid cellular uptake has not been discovered yet.

(2) Inhibitors of FAAH, such as URB-597, OL-135, BMS-1, SA-47, PF-750 and *N*-arachidonoyl-serotonin (which also antagonizes TRPV1 receptors). More and more such inhibitors are being developed, and they include both reversible and irreversible inhibitors (see [111,112] for reviews). Possible therapeutic applications for such compounds are hypertension, glaucoma, emesis, locomotor impairment in Parkinson's disease, anxiety, depression, gastrointestinal and hepatic disorders, ulcerative colitis, colorectal cancer and neuropathic and inflammatory pain [88,111].

(3) Inhibitors of MAGL, such as URB602 and *N*-arachidonoyl-maleimide, or the more potent and recently discovered OMDM169 [113] and JZL184 [114]. Therapeutic drugs developed from these compounds are likely to have the same indications as FAAH inhibitors, and possibly less complications due to the fact that, as opposed to FAAH inhibition, MAGL inhibition does not cause elevation of the levels of non-endocannabinoid molecules.

(4) Dual CB_1/CB_2 agonists, such as WIN-55,512-2, CP-55940 and HU-210. These compounds have been, and still are, very useful in pharmacological studies on the function of cannabinoid receptors, but are unlikely to generate new therapeutic drugs.

(5) Anandamide analogues that are more metabolically stable than the parent compound and more suitable for in vivo studies, such as methanandamide and metfluoroanandamide. These compounds are very useful for studies in biological systems that contain high levels of FAAH, but have been reported to also activate TRPV1 receptors.

(6) Selective CB_1 agonists, such as arachidonoylchloroethanolamide and arachidonoyl-cyclopropylamide (ACEA). Such compounds have been very useful in both *in vitro* and in vivo studies to distinguish the effects of CB_1 receptor activation from those associated to CB_2 receptors.

(7) Selective CB_2 agonists, such as HU-308, JWH-015, JWH-133 and AM1241. Such compounds have been very useful in both *in vitro* and in vivo studies to distinguish the effects of CB_2 receptors from those of associated to CB_1 receptors. They might also represent important templates for the development of non-psychotropic anti-inflammatory and analgesic drugs.

(8) Relatively selective inhibitors of 2-AG biosynthesis, such as O-3640, O-3841 [115], OMDM188 [116] and O-5596 [117]. Apart from having been very useful to establish the direct role of 2-AG, rather than anandamide, in retrograde signalling [105,118] and in slow self-inhibition [119], some of these compounds might serve as templates for the development of anti-obesity agents [117].

(9) Selective antagonists/inverse agonists for CB_1 receptors, such as SR141716A (rimonabant), SR147778 (surinabant), AM251, AM281, MK-0363 (Taranabant), LY320135, CP-945598 and AVE1625. Some of these compounds have already found clinical use as anti-obesity agents as well as against metabolic disorders such as dyslipidemia and type 2 diabetes, although their use in these pathologies has been discontinued due to their psychiatric side effects (namely anxiety and depression). Other possible uses might be against steatosis and steatohepatitis, nicotine and alcohol abuse, relapse of heroin and cocaine abuse, hypotension, cardiopathies, encephalopathy and liver fibrosis in cirrhosis, Parkinson's and Alzheimer's disease, schizophrenia and osteoporosis. Efforts are ongoing to develop non-brain-permeant CB_1 receptor antagonists/inverse agonists, which should be devoid of the central side effects of rimonabant and taranabant and still useful against some metabolic disorders [120].

(10) Neutral CB_1 antagonists, such as AM4113. These compounds would be more useful than inverse agonists as pharmacological tools as they would produce effects only in the presence of elevated endocannabinoid levels.

(11) Selective CB_2 antagonist/inverse agonists, i.e. SR144528, AM630 and JTE907. Some of these compounds are being developed as anti-inflammatory agents [88].

(12) Allosteric modulators of CB_1 receptors, including Org27596, Org29647 and PSNCBAM-1 [121]. These compounds enhance the affinity of CB_1 receptor agonists but reduce their efficacy, and might, therefore, find application in the same pathological conditions as CB_1 antagonists/inverse agonists.

The chemical structures of the most widely used of these compounds is shown in Fig. 2.

4. Conclusions

Perhaps one of the most intriguing "control devices" in mammals, the endocannabinoid system is emerging as a key player in several physiological and pathological mechanisms, in both central and peripheral tissues. As such, this system is likely to lead in the future to the development of new therapeutic tools targeting disorders that have been so far poorly managed in the clinical practice. Numerous examples exist of how "direct" or "indirect" activation of cannabinod receptors can either counteract or contribute to the symptoms and/or progress of different pathologies. Furthermore, endocannabinoids seem sometimes to participate with opposing effects – and, correspondingly, molecules that either reduce or enhance endocannabinoid tone both produce beneficial effects – in different phases of the same disease [88]. Therefore, the most challenging future task for the pharmaceutical chemist and the pharmacologist will be to devise ways to target this pleiotropic and "plastic" system in a selective, and hence, safe way, thus obtaining therapeutic drugs with more and more favourable benefit-to-risk profiles.

Acknowledgement

The author is grateful to Dr. Fabiana Piscitelli for her help with the preparation of the manuscript.

References

[1] Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–7.
[2] Devane WA, Dysarz III FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605–13.
[3] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–4.
[4] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.
[5] Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9.
[6] Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 1995;50:83–90.
[7] Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 1995;215:89–97.

[8] Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, et al. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. Proc Natl Acad Sci USA 2001;98:3662–5.

[9] Bisogno T, Melck D, Bobrov MYu, Gretskaya NM, Bezuglov VV, De Petrocellis L, et al. N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J 2000;351:817–24.

[10] Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, et al. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci USA 2002;99:8400–5.

[11] Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J, et al. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB_1 receptor. J Pharmacol Exp Ther 2002;301:1020–4.

[12] Heimann AS, Gomes I, Dale CS, Pagano RL, Gupta A, de Souza LL, et al. Hemopressin is an inverse agonist of CB_1 cannabinoid receptors. Proc Natl Acad Sci USA 2007;104:20588–93.

[13] Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 1994;372:686–91.

[14] Di Marzo V, De Petrocellis L, Sugiura T, Waku K. Potential biosynthetic connections between the two cannabimimetic eicosanoids, anandamide and 2-arachidonoyl-glycerol, in mouse neuroblastoma cells. Biochem Biophys Res Commun 1996;227:281–8.

[15] Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, Di Marzo V. Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. Biochem J 1997;322:671–7.

[16] Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature 1997;388:773–8.

[17] Cadas H, di Tomaso E, Piomelli D. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. J Neurosci 1997;17:1226–42.

[18] Bisogno T, Melck D, De Petrocellis L, Di Marzo V. Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J Neurochem 1999;72:2113–9.

[19] Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83–7.

[20] Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 1997;272:27218–23.

[21] Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 2002;99:10819–24.

[22] Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 2003;163:463–8.

[23] Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 2004;279:5298–305.

[24] Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 1997;277:1094–7.

[25] Beltramo M, Piomelli D. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. Neuroreport 2000;11:1231–5.

[26] Bisogno T, Maccarrone M, De Petrocellis L, Jarrahian A, Finazzi-Agrò A, Hillard C, et al. The uptake by cells of 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors. Eur J Biochem 2001;268:1982–9.

[27] Glaser ST, Abumrad NA, Fatade F, Kaczocha M, Studholme KM, Deutsch DG. Evidence against the presence of an Anandamide transporter. Proc Natl Acad Sci USA 2003;100:4269–74.

[28] Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. Science 2002;298:1793–6.

[29] Maione S, Morera E, Marabese I, Ligresti A, Luongo L, Ortar G, et al. Antinociceptive effects of tetrazole inhibitors of endocannabinoid inactivation: cannabinoid and non-cannabinoid receptor-mediated mechanisms. Br J Pharmacol 2008;155:775–82.

[30] Fowler CJ, Ghafouri N. Does the hydrolysis of 2-arachidonoylglycerol regulate its cellular uptake? Pharmacol Res 2008;58:72–6.

[31] Ortega-Gutiérrez S, Hawkins EG, Viso A, López-Rodríguez ML, Cravatt BF. Comparison of anandamide transport in FAAH wild-type and knockout neurons: evidence for contributions by both FAAH and the CB_1 receptor to anandamide uptake. Biochemistry 2004;43:8184–90.

[32] Leung D, Saghatelian A, Simon GM, Cravatt BF. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. Biochemistry 2006;45:4720–6.

[33] Simon GM, Cravatt BF. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. J Biol Chem 2006;281:26465–72.

[34] Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA 2006;103:13345–50.

[35] Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, et al. Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology 2008;54:1–7.

[36] Sun YX, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, et al. Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. Biochem J 2004;380:749–56.

[37] Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 2007;14:1347–56.

[38] Di Marzo V, Maccarrone M. FAAH and anandamide: is 2-AG really the odd one out? Trends Pharmacol Sci 2008;29:229–33.

[39] Mulder AM, Cravatt BF. Endocannabinoid metabolism in the absence of fatty acid amide hydrolase (FAAH): discovery of phosphorylcholine derivatives of N-acyl ethanolamines. Biochemistry 2006;45:11267–77.

[40] Kozak KR, Marnett LJ. Oxidative metabolism of endocannabinoids. Prostaglandins Leukot Essent Fatty Acids 2002;66:211–20.

[41] Woodward DF, Liang Y, Krauss AH. Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol 2008;153:410–9.

[42] Matias I, Chen J, De Petrocellis L, Bisogno T, Ligresti A, Fezza F, et al. Prostaglandin ethanolamides (prostamides): in vitro pharmacology and metabolism. J Pharmacol Exp Ther 2004;309:745–57.

[43] Liang Y, Woodward DF, Guzman VM, Li C, Scott DF, Wang JW, et al. Identification and pharmacological characterization of the prostaglandin FP receptor and FP receptor variant complexes. Br J Pharmacol 2008;154:1079–93.

[44] Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. Br J Pharmacol 2000;275:33744–9.

[45] Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R, et al. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 2002;277:44877–85.

[46] Nirodi CS, Crews BC, Kozak KR, Morrow JD, Marnett LJ. The glyceryl ester of prostaglandin E2 mobilizes calcium and activates signal transduction in RAW264, 7 cells. Proc Natl Acad Sci USA 2004;101:1840–5.

[47] Vila A, Rosengarth A, Piomelli D, Cravatt B, Marnett LJ. Hydrolysis of prostaglandin glycerol esters by the endocannabinoid-hydrolyzing enzymes, monoacylglycerol lipase and fatty acid amide hydrolase. Biochemistry 2007;46:9578–85.

[48] Ueda N, Yamamoto K, Yamamoto S, Tokunaga T, Shirakawa E, Shinkai H, et al. Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. Biochim Biophys Acta 1995;1254:127–34.

[49] Edgemond WS, Hillard CJ, Falck JR, Kearn CS, Campbell WB. Human platelets and polymorphonuclear leukocytes synthesize oxygenated derivatives of arachidonylethanolamide (anandamide): their affinities for cannabinoid receptors and pathways of inactivation. Mol Pharmacol 1998;54:180–8.

[50] van der Stelt M, van Kuik JA, Bari M, van Zadelhoff G, Leeflang BR, Veldink GA, et al. Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. J Med Chem 2002;45:3709–20.

[51] Bornheim LM, Kim KY, Chen B, Correia MA. Microsomal cytochrome P450-mediated liver and brain anandamide metabolism. Biochem Pharmacol 1995;50:677–86.

[52] Snider NT, Sikora MJ, Sridar C, Feuerstein TJ, Rae JM, Hollenberg PF. The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6. J Pharmacol Exp Ther 2008;327:538–45.

[53] Howlett AC. Cannabinoid receptor signaling. Handb Exp Pharmacol 2005;168:53–79.

[54] Twitchell W, Brown S, Mackie K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J Neurophysiol 1997;781:43–50.

[55] Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR. Cannabinoid CB_1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. Am J Physiol 1999;276:2085–93.

[56] Sugiura T, Kodaka T, Kondo S, Tonegawa T, Nakane S, Kishimoto S, et al. 2-Arachidonoylglycerol, a putative endogenous cannabinoid receptor ligand, induces rapid, transient elevation of intracellular free Ca^{2+} in neuroblastoma × glioma hybrid NG108-15 cells. Biochem Biophys Res Commun 1996;229:58–64.

[57] Ho BY, Uezono Y, Takada S, Takase I, Izumi F. Coupling of the expressed cannabinoid CB_1 and CB_2 receptors to phospholipase C and G protein-coupled inwardly rectifying K^+ channels. Receptors Channels 1999;6:363–74.

[58] Lauckner JE, Hille B, Mackie K. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB_1 receptor coupling to Gq/11 G proteins. Proc Natl Acad Sci USA 2005;102:19144–9.

[59] De Petrocellis L, Marini P, Matias I, Moriello AS, Starowicz K, Cristino L, et al. Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. Exp Cell Res 2007;313:2993–3004.

[60] Gómez del Pulgar T, Velasco G, Guzmán M. The CB_1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. Biochem J 2000;347:369–73.

[61] Sánchez MG, Ruiz-Llorente L, Sánchez AM, Díaz-Laviada I. Activation of phosphoinositide 3-kinase/PKB pathway by CB(1) and CB(2) cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. Cell Signal 2003;15:851–9.

[62] Ellert-Miklaszewska A, Kaminska B, Konarska L. Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. Cell Signal 2005;17:25–37.

[63] Ozaita A, Puighermanal E, Maldonado R. Regulation of PI3K/Akt/GSK-3 pathway by cannabinoids in the brain. J Neurochem 2007;102:1105–14.
[64] Esposito I, Proto MC, Gazzerro P, Laezza C, Miele C, Alberobello AT, et al. The cannabinoid CB_1 receptor antagonist rimonabant stimulates 2-deoxyglucose uptake in skeletal muscle cells by regulating the expression of phosphatidylinositol-3-kinase. Mol Pharmacol 2008;74:1678–86.
[65] Stefano GB, Esch T, Cadet P, Zhu W, Mantione K, Benson H. Endocannabinoids as autoregulatory signaling molecules: coupling to nitric oxide and a possible association with the relaxation response. Med Sci Monit 2003;9:RA63–75.
[66] Sergeeva OA, Doreulee N, Chepkova AN, Kazmierczak T, Haas HL. Long-term depression of cortico-striatal synaptic transmission by DHPG depends on endocannabinoid release and nitric oxide synthesis. Eur J Neurosci 2007;26:1889–94.
[67] Lépicier P, Lagneux C, Sirois MG, Lamontagne D. Endothelial CB_1-receptors limit infarct size through NO formation in rat isolated hearts. Life Sci 2007;81:1373–80.
[68] Newman Z, Malik P, Wu TY, Ochoa C, Watsa N, Lindgren C. Endocannabinoids mediate muscarine-induced synaptic depression at the vertebrate neuromuscular junction. Eur J Neurosci 2007;25:1619–30.
[69] Jones JD, Carney ST, Vrana KE, Norford DC, Howlett AC. Cannabinoid receptor-mediated translocation of NO-sensitive guanylyl cyclase and production of cyclic GMP in neuronal cells. Neuropharmacology 2008;54(January (1)):23–30.
[70] Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sørgård M, Di Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999;400:452–7.
[71] Starowicz K, Nigam S, Di Marzo V. Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 2007;114:13–33.
[72] Pacher P, Bátkai S, Kunos G. Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. J Physiol 2004;558:647–57.
[73] De Petrocellis L, Starowicz K, Moriello AS, Vivese M, Orlando P, Di Marzo V. Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB(1) receptors and endovanilloids. Exp Cell Res 2007;313:1911–20.
[74] De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J Pharmacol Exp Ther 2008;325:1007–15.
[75] Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 2007;152:1092–101.
[76] Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc Natl Acad Sci USA 2008;105:2699–704.
[77] Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, et al. Integrin clustering enables anandamide-induced Ca^{2+} signaling in endothelial cells via GPR55 by protection against CB_1-receptor-triggered repression. J Cell Sci 2008;121:1704–17.
[78] Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun 2007;362:928–34.
[79] Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ. The GPR55 ligand L-{alpha}-lysophosphatidylinositol promotes RhoA-dependent Ca^{2+} signaling and NFAT activation. FASEB J 2009;23:183–93.
[80] Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, et al. Endocannabinoid activation at hepatic CB_1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 2005;115:1298–305.
[81] Ashton JC, Glass M. The cannabinoid CB_2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol 2007;5:73–80.
[82] Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, et al. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. Science 2005;310:329–32.
[83] Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, et al. Brain neuronal CB_2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. PLoS ONE 2008;3:e1640.
[84] Guzmán M, Sánchez C, Galve-Roperh I. Cannabinoids and cell fate. Pharmacol Ther 2002;95:175–84.
[85] Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 2004;3:771–84.
[86] Pertwee RG. Pharmacological actions of cannabinoids. Handb Exp Pharmacol 2005;(168):1–51.
[87] Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006;58:389–462.
[88] Di Marzo V. Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov 2008;7:438–55.
[89] Pertwee RG. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J 2005;7:E625–54.
[90] Di Marzo V, Petrosino S. Endocannabinoids and the regulation of their levels in health and disease. Curr Opin Lipidol 2007;18:129–40.
[91] Bisogno T, Di Marzo V. Short- and long-term plasticity of the endocannabinoid system in neuropsychiatric and neurological disorders. Pharmacol Res 2007;56:428–42.
[92] Moreira FA, Lutz B. The endocannabinoid system: emotion, learning and addiction. Addict Biol 2008;13:196–212.
[93] Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med 2008;14:923–30.
[94] Mackie K. Signaling via CNS cannabinoid receptors. Mol Cell Endocrinol 2008;286:S60–5.
[95] Wang H, Dey SK, Maccarrone M. Jekyll and Hyde: two faces of cannabinoid signaling in male and female fertility. Endocr Rev 2006;27:427–48.
[96] Jeong WI, Osei-Hyiaman D, Park O, Liu J, Bátkai S, Mukhopadhyay P, et al. Paracrine activation of hepatic CB_1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. Cell Metab 2008;7:227–35.
[97] Egertová M, Giang DK, Cravatt BF, Elphick MR. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB_1 receptor in rat brain. Proc Biol Sci 1998;265:2081–5.
[98] Nyilas R, Dudok B, Urbán GM, Mackie K, Watanabe M, Cravatt BF, et al. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. J Neurosci 2008;28:1058–63.
[99] Egertová M, Simon GM, Cravatt BF, Elphick MR. Localization of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: a new perspective on N-acylethanolamines as neural signaling molecules. J Comp Neurol 2008;506:604–15.
[100] Cristino L, Starowicz K, De Petrocellis L, Morishita J, Ueda N, Guglielmotti V, et al. Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. Neuroscience 2008;151:955–68.
[101] Mezey E, Tóth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, et al. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci USA 2000;97:3655–60.
[102] Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 2006;139:1405–15.
[103] Marinelli S, Di Marzo V, Florenzano F, Fezza F, Viscomi MT, van der Stelt M, et al. N-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. Neuropsychopharmacology 2007;32:298–308.
[104] Di Marzo V, Cristino L. Why endocannabinoids are not all alike. Nat Neurosci 2008;11:124–6.
[105] Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, Musella A, et al. Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. Nat Neurosci 2008;11:152–9.
[106] van der Stelt M, Trevisani M, Vellani V, De Petrocellis L, Schiano Moriello A, Campi B, et al. Anandamide acts as an intracellular messenger amplifying Ca^{2+} influx via TRPV1 channels. EMBO J 2005;24:3026–37.
[107] Fowler CJ. "The tools of the trade"—an overview of the pharmacology of the endocannabinoid system. Curr Pharm Des 2008;14:2254–65.
[108] Schiano Moriello A, Balas L, Ligresti A, Cascio MG, Durand T, Morera E, et al. Development of the first potential covalent inhibitors of anandamide cellular uptake. J Med Chem 2006;49:2320–32.
[109] Ligresti A, Cascio MG, Pryce G, Kulasegram S, Beletskaya I, De Petrocellis L, et al. New potent and selective inhibitors of anandamide reuptake with antispastic activity in a mouse model of multiple sclerosis. Br J Pharmacol 2006;147:83–91.
[110] Ortar G, Cascio MG, Moriello AS, Camalli M, Morera E, Nalli M, et al. Carbamoyl tetrazoles as inhibitors of endocannabinoid inactivation: a critical revisitation. Eur J Med Chem 2008;43:62–72.
[111] Fezza F, De Simone C, Amadio D, Maccarrone M. Fatty acid amide hydrolase: a gate-keeper of the endocannabinoid system. Subcell Biochem 2008;49:101–32.
[112] Seierstad M, Breitenbucher JG. Discovery and development of fatty acid amide hydrolase (FAAH) inhibitors. J Med Chem 2008 [Epub ahead of print].
[113] Bisogno T, Ortar G, Petrosino S, Morera E, Palazzo E, Nalli M, et al. Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity in vivo. Biochim Biophys Acta 2009;1791:53–60.
[114] Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol 2009;5:37–44.
[115] Bisogno T, Cascio MG, Saha B, Mahadevan A, Urbani P, Minassi A, et al. Development of the first potent and specific inhibitors of endocannabinoid biosynthesis. Biochim Biophys Acta 2006;1761:205–12.
[116] Ortar G, Bisogno T, Ligresti A, Morera E, Nalli M, Di Marzo V. Tetrahydrolipstatin analogues as modulators of endocannabinoid 2-arachidonoylglycerol metabolism. J Med Chem 2008;51:6970–9.
[117] Bisogno T, Burston JJ, Rai R, Allarà M, Saha B, Mahadevan A, et al. Synthesis and pharmacological activity of a potent inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol. ChemMedChem; in press, 2009 Mar 5. [Epub ahead of print].
[118] Melis M, Pillolla G, Bisogno T, Minassi A, Petrosino S, Perra S, et al. Protective activation of the endocannabinoid system during ischemia in dopamine neurons. Neurobiol Dis 2006;24:15–27.
[119] Marinelli S, Pacioni S, Bisogno T, Di Marzo V, Prince DA, Huguenard JR, et al. The endocannabinoid 2-arachidonoylglycerol is responsible for the slow self-inhibition in neocortical interneurons. J Neurosci 2008;28:13532–41.
[120] Kunos G, Osei-Hyiaman D, Bátkai S, Sharkey KA, Makriyannis A. Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? Trends Pharmacol Sci 2009;30:1–7.
[121] Ross RA. Allosterism and cannabinoid CB(1) receptors: the shape of things to come. Trends Pharmacol Sci 2007;28(11):567–72.