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Review

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

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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

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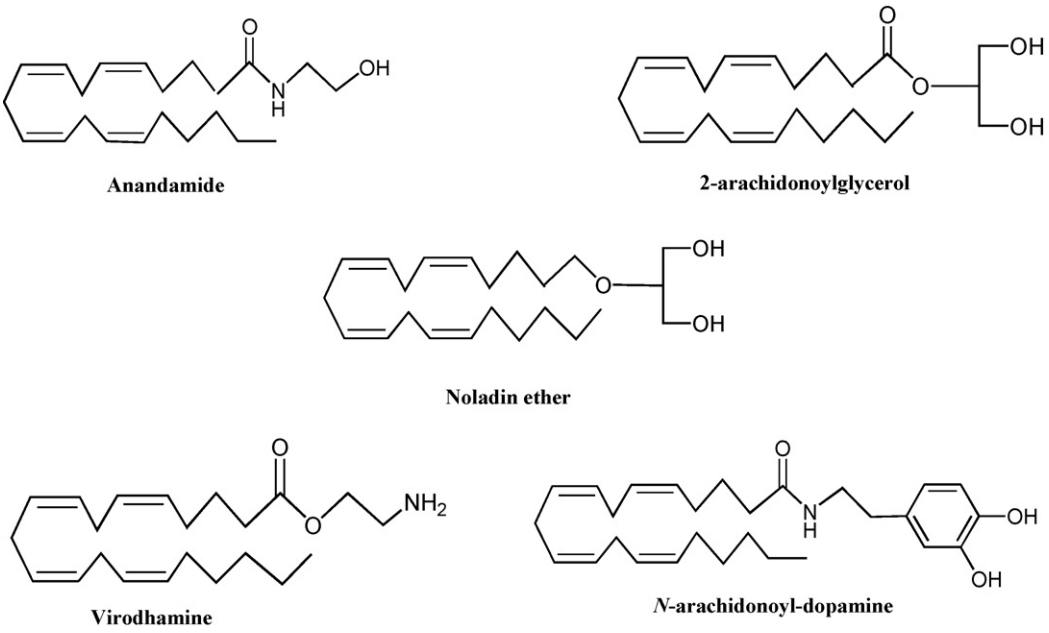

Fig. 1. Chemical structures of the proposed endocannabinoids.

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

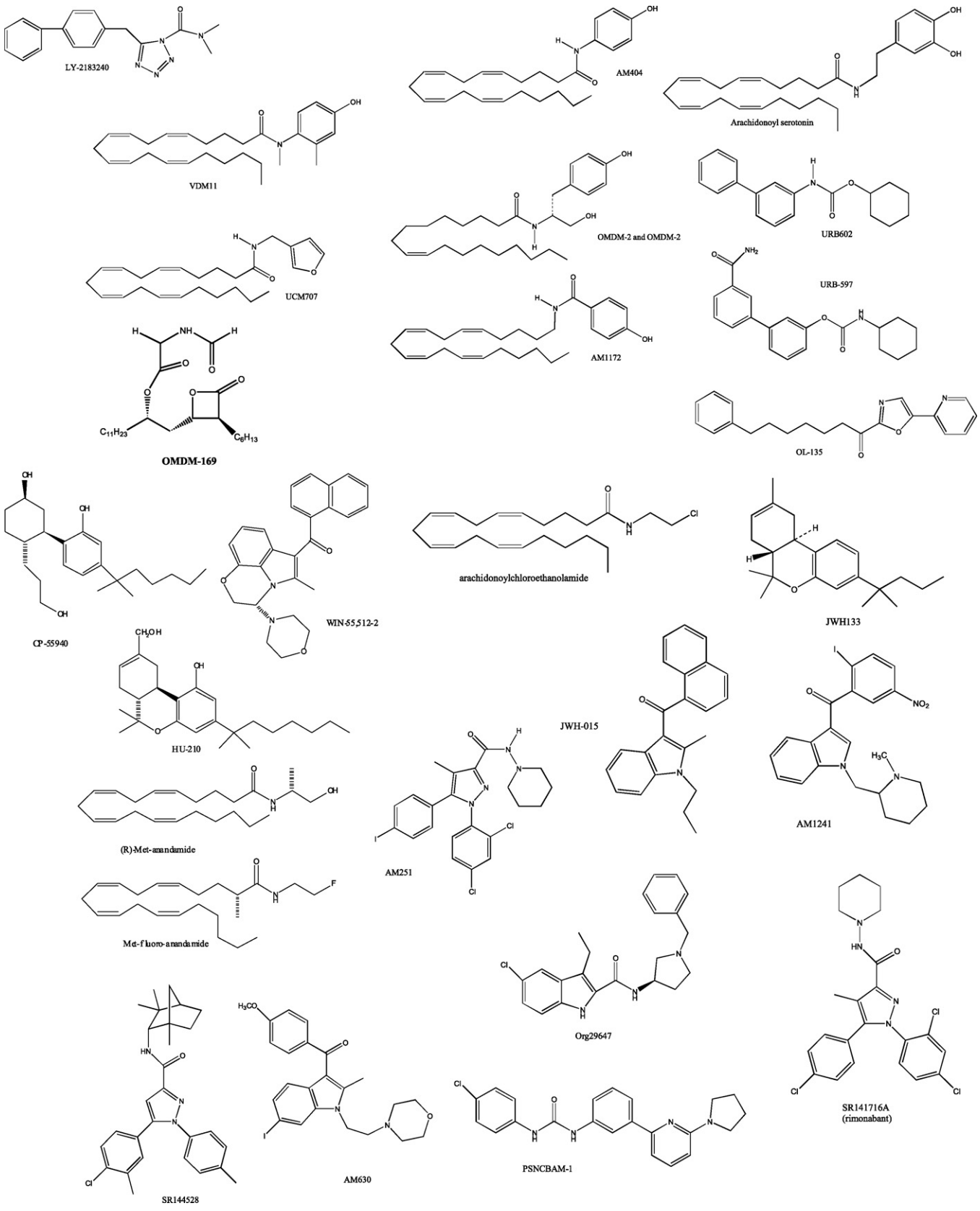

Fig. 2. Chemical structures of some of the pharmacological tools used to investigate the endocannabinoid system.

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.

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