Endocannabinoids and the regulation of their levels in health and disease

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Purpose of review

Endocannabinoids are defined as endogenous agonists of cannabinoid receptors, that is, of the two G-protein-coupled receptors for the *Cannabis* psychoactive principle Δ^9-tetra-hydrocannabinol. Two such endogenous mediators have been most thoroughly studied so far: anandamide and 2-arachidonoylglycerol. Here we review the mechanisms for the regulation of their levels under physiological and pathological conditions, and recent findings on their role in disease.

Recent findings

It is becoming increasingly clear that, although both anandamide and 2-arachidonoyl-glycerol are produced and degraded 'on demand', the levels of these two compounds appear to be regulated in different, and sometimes even opposing, ways, often using redundant molecular mechanisms. Alterations of endocannabinoid levels have been found in both animal models of pain, neurological and neurodegenerative states, gastrointestinal disorders and inflammatory conditions, and in blood, cerebrospinal fluid and bioptic samples from patients with various diseases.

Summary

Endocannabinoid levels appear to be transiently elevated as an adaptive reaction to re-establish normal homeostasis when this is acutely and pathologically perturbed. In some chronic conditions, however, this system also contributes to the progress or symptoms of the disorder. As a consequence, new therapeutic drugs are being designed from both stimulants and blockers of endocannabinoid action.

Keywords

2-arachidonoylglycerol, anandamide, cannabinoid, gastrointestinal disorders, inflammation, neurodegeneration

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Abbreviations

2-AG	2-arachidonoyl-glycerol
AEA	*N*-arachidonoyl-ethanolamine
CNS	central nervous system
DAG	diacylglycerol
FAAH	fatty acid amide hydrolase
NAE	*N*-acylethanolamine
NAPE	*N*-acylphosphatidyl-ethanolamine
NAPE-PLD	NAPE-selective phospholipase D
NArPE	*N*-arachidonoylphosphatidylethanolamine
PLC	phospholipase C

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Introduction

Two subtypes of G-protein-coupled receptors for the *Cannabis* psychotropic component, Δ^9-tetrahydrocannabinol (THC), have been cloned to date: the cannabinoid CB_1 and CB_2 receptors [1]; and several types of endogenous agonists for these cannabinoid receptors have been also identified (Fig. 1). These compounds, originally named endocannabinoids [2], are derived from arachidonic acid, and *N*-arachidonoyl-ethanolamine (AEA, anandamide) [3], and 2-arachidonoyl-glycerol (2-AG) [4,5] have been most studied. Here we review the mechanisms for the regulation of endocannabinoid levels under physiological and pathological conditions.

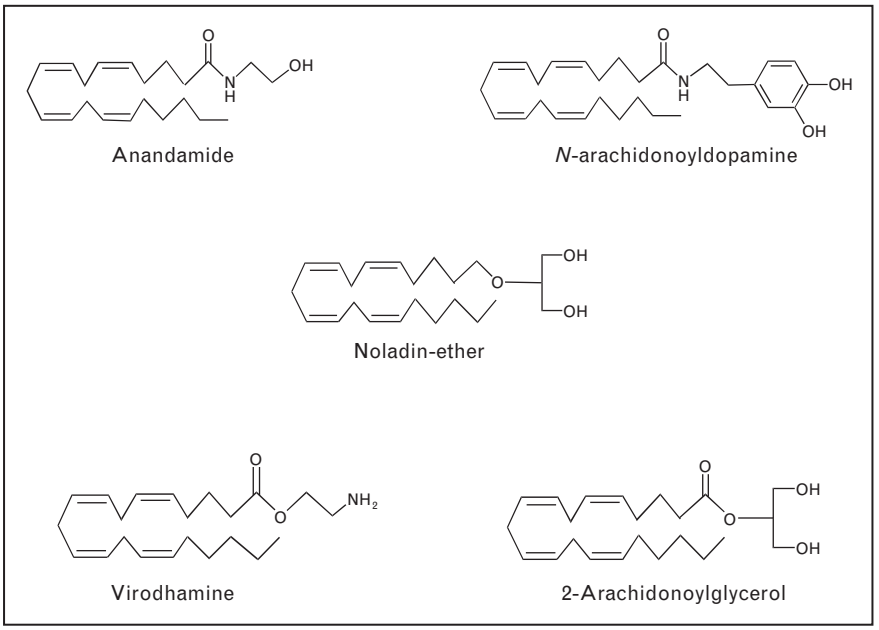
Biochemical regulation of endocannabinoid levels

AEA and 2-AG are biosynthesized 'on demand' and released from cells immediately after their production. These events are triggered by the enhancement of intracellular calcium concentrations that follows cell depolarization or the mobilization of intracellular calcium stores subsequent to stimulation of G_q/G_{11} protein-coupled receptors. Accordingly, the enzymes catalysing AEA and 2-AG biosynthesis are calcium-sensitive. Since their direct biosynthetic precursors belong to two families of lipids, the *N*-acylphosphatidyl-ethanolamines (NAPEs) and the diacylglycerols (DAGs), respectively, and the biosynthesizing enzymes are not very selective for any member of these families, AEA and 2-AG are usually found together with their congeners, the *N*-acylethanolamines (NAEs) and the 2-acylglycerols, respectively. Also the degrading enzymes for AEA and 2-AG recognize as substrates other NAEs and 2-acylglycerols, respectively.

Endocannabinoid biosynthesis

The AEA precursor, *N*-arachidonoylphosphatidylethanolamine (NArPE) [6], is produced by transferring the

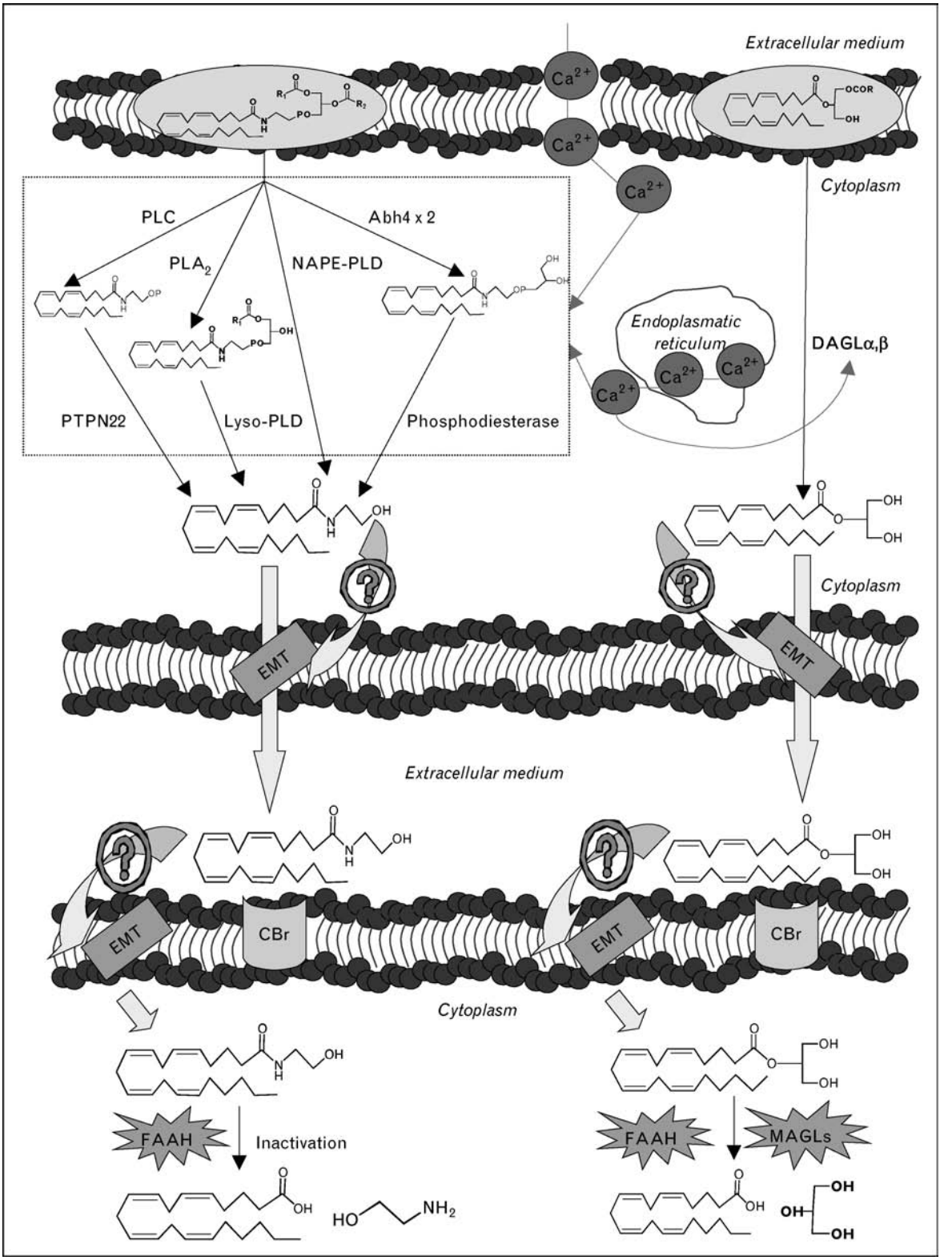
Figure 1 Chemical structures of the putative endocannabinoids identified to date



little arachidonic acid esterified on the *sn*-1 position of other phospholipids to the nitrogen atom of phosphatidylethanolamine, via an as-yet unidentified *N*-acyl-transferase. The enzyme catalysing the one-step conversion of NAPEs into the corresponding NAEs, termed NAPE-selective phospholipase D (NAPE-PLD) [7], is enzymatically distinct from other phospholipase D enzymes; stimulated by calcium; and almost equally efficacious with most NAPEs, and hence responsible for the formation of other NAEs that, like the C16 : 0, C18 : 0 and C18 : 1 congeners, possess pharmacological activity independent of cannabinoid receptors. NAPE-PLD is a member of the β-lactamase, zinc-metallo-hydrolase family of enzymes [7]. Its overexpression in cells leads to higher cellular levels of AEA and correspondingly lower levels of NArPE, thus supporting its role in the biosynthesis of this endocannabinoid [8•]. NAPE-PLD is a determinant of uterine anandamide levels during mouse pregnancy, and its expression correlates spatially and temporally with its activity and with AEA levels, which in turn control embryo implantation [9•]. Three more pathways have been identified, however, that transform, via multistep reactions, NArPE into AEA. One route converts NAPEs into 2-lyso-NAPEs via the action of a groupIB soluble phospholipase A_2, and then 2-lyso-NAPEs into NAEs via a selective lysophospholipase D [10]. A phospholipase C (PLC)-dependent pathway for NArPE conversion to AEA via phospho-AEA was recently suggested to occur in macrophages, and to involve PTPN22, a protein tyrosine phosphatase, as the enzyme catalysing phospho-AEA hydrolysis [11••]. Finally, in mouse brain homogenates, NArPE is a substrate for α/β-hydrolase 4 (Abh4), acting two times as a phospholipase B/lysophospholipase for the formation of glycerophospho-AEA, which is then converted into AEA by a specific phosphodiesterase (Fig. 2) [12•]. These redundant AEA biosynthetic routes explain why NAPE-PLD deficient mice contain low levels of saturated NAEs, but not AEA [12•], and might obstruct the development of selective inhibitors of AEA biosynthesis.

2-AG exhibits higher selectivity and efficacy for CB_1 and CB_2 receptors than AEA, which interacts also with non-cannabinoid receptor targets. Therefore, it is not surprising that the levels of the two compounds are regulated in different ways. 2-Arachidonate-containing DAGs are in most cases the biosynthetic precursors of 2-AG, and are produced from the hydrolysis either of phosphoinositol-bis-phosphate (PIP_2), catalysed by the PIP_2-selective phospholipase C (PLCβ), or of phosphatidic acid, catalysed by the phosphatidic acid-selective phosphohydrolase [13,14,15•]. DAGs are then converted into 2-AG by the action of two plasma membrane *sn*-1-selective DAG lipases, DAGLα and DAGLβ, recently cloned (Fig. 2) [16]. These two enzymes contain the typical lipase-3 and Ser-lipase signature sequences; two highly conserved amino acid residues within the Ser-lipase signature sequence, Ser443 and Asp495, necessary for enzymatic activity; and four hydrophobic, and possibly *trans*-membrane, domains near their N-terminus. Both enzymes are stimulated by calcium and inhibited by glutathione, and although they exhibit strong selectivity for DAGs over phospholipids, monoacyloglycerides, triacylglycerols and fatty acid amides, they do not appear to prefer DAGs with

Figure 2 Proteins and pathways for the biosynthesis and degradation of endocannabinoids



2-AG, 2-arachidonoyl glycerol; Abh4, α/β-hydrolase 4; CBr, cannabinoid receptor; DAGL, *sn*-1-selective diacylglycerol lipase; EMT, putative endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; lyso-PLD, lysophospholipase D; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidyl-ethanolamine-selective phospholipase D; PLA_2, phospholipase A_2; PLC, phospholipase C; PTPN22, protein tyrosine phosphatase N22.

any particular fatty acyl chain in the 2 or *sn*-1 position. Although DAGLα is more abundant in the adult brain, and DAGLβ in the developing brain, the cellular localization of both enzymes shifts during brain development, as they are co-localized with CB_1 in neuronal axons of the perinatal nervous system, and 'move' to postsynaptic neurons in the adult brain [16]. Indeed, DAGLα localizes to the postsynaptic dendritic spines establishing synapses with CB_1 expressing axons [17••,18••], thus supporting the proposed roles for 2-AG as a retrograde messenger of synaptic plasticity in the adult brain [19,20••].

The role of the two DAGLs in the control of 2-AG levels is confirmed by the fact that their expression/activity reflects the tissue concentrations of 2-AG under certain physiopathological concentrations: during diurnal variations of 2-AG levels [21], following induction of neurotoxicity with a β-amyloid peptide [22•], or when 2-AG uterine concentrations are upregulated in mice with defective leptin signalling [23]. Moreover, pharmacological inhibition of the DAGLs with tetrahydrolipostatin, a potent but unselective DAGL inhibitor, or O-3640 and O-3841, two selective but less cell-permeable compounds [24••], blocks CB_1-receptor-mediated cellular effects such as FGF-induced axonal sprouting in the developing brain [16]; depolarization-induced suppression of excitation in adult dopaminergic neurons and protection of these neurons from ischaemia [25,19]; depolarization-induced suppression of inhibition in Purkinje's cells [20••]; and long-lasting self-inhibition of neocortical interneurons [26].

The PLCβ–DAG–DAGL pathway does not always underlie 2-AG biosynthesis [27•,28•]. Transgenic mice lacking G_q/G_{11} proteins that mediate metabotropic receptor-induced activation of PLCβ do not exhibit reduced brain levels of this endocannabinoid [29••]. It is possible that most of the relatively high basal levels of 2-AG are not used to activate cannabinoid receptors (the tissue concentration of this compound in the rat brain approximates 5 μM, and would cause a permanent activation of CB_1 receptors), and are formed via biosynthetic precursors other than DAGs or through the action of PLC isoforms different from PLCβ. On the other hand, if mice are stimulated with kainic acid, which causes neuronal depolarization and calcium influx, their brains cannot synthesize 'on demand' 2-AG in the absence of G_q/G_{11} proteins, and subsequently cannot protect themselves against kainate-induced excitotoxicity [29••]. This is in agreement with the finding of PLCβ as a coincidence detector directing extracellular and intracellular calcium mobilization into the formation of 2-AG [30•]. Indeed, unlike AEA, both intracellular calcium and PLCβ-derived biosynthetic precursors (i.e. DAGs) are necessary for the formation of that population of 2-AG acting as a brain endocannabinoid, at least under certain conditions.

On the other hand, AEA is under the control of G_q/G_{11} proteins, since transgenic mice lacking these two proteins exhibit significantly reduced AEA brain levels [29••]. By contrast, when these animals are challenged with kainic acid, their brains still produce AEA. Thus, depending on the conditions, AEA, unlike 2-AG, can be synthesized following mobilization of either intracellular or extracellular calcium alone, possibly because PLCβ is not required to produce NArPE nor to convert this compound into AEA in the central nervous system (CNS). These data emphasize how the levels of the two main endocannabinoids are regulated in different ways.

Endocannabinoid degradation

AEA is inactivated through the hydrolysis to arachidonic acid and ethanolamine. The enzyme catalysing this reaction [31] also recognizes as substrates other long chain fatty acid amides, including other NAEs, fatty acid primary amides, *N*-acylaminoacids and *N*-acyltaurines [32,33]; hence the name 'fatty acid amide hydrolase' (FAAH) originally given to this protein, which, however, also efficiently catalyses the hydrolysis of fatty acyl esters, including 2-AG (Fig. 2). The several important structural features of FAAH, revealed also by site-directed mutagenesis and crystallographic X-ray studies, were recently discussed in a comprehensive review [34], and will not be described here. The physiopathological role of FAAH is now being currently investigated thanks to the study of the phenotype of transgenic mice lacking the enzyme, the 'FAAH-knockout mice', of which there are now available peripheral tissue specific-knockout mutants [35]; the design of specific FAAH inhibitors suitable for use *in vivo* [36–38]; immunohistochemical studies describing the tissue and cellular distribution of the enzyme and its relationship with CB_1 receptor distribution [39]; the identification of the promoter region of the *Faah* gene and of its regulation by several transcription factors and hormones [40,41]; and the identification of *Faah* gene polymorphisms associated with disorders such as obesity, propensity to drug addiction and schizophrenia [42••,43,44]. At least in the case of AEA and fatty acid amides, there is little doubt that FAAH is the major, if not the only, degrading enzyme in rodents, because AEA levels are elevated following administration of FAAH inhibitors to rodents [36,37,45], and in most tissues of FAAH-deficient mice, or only in the periphery in the case of the peripheral organ-specific $FAAH^{-/-}$ mice [35]. The recent cloning of FAAH-2, however, an FAAH isoform with only 20% homology to FAAH, occurring in several mammalian species, including primates, but absent in rodents [46•], opens the possibility that there is redundancy in NAE degradation as with NAE

biosynthesis. Whereas FAAH-2 is very efficacious with monounsaturated fatty acid amides, however, it shows little efficacy at hydrolysing AEA, whereas the recently cloned '*N*-acylethanolamine-hydrolysing acid amidase' [47] is more specific for long chain saturated NAEs. In mice, where FAAH-2 is not expressed, pharmacological or genetical inactivation of FAAH causes AEA upregulation in the liver and intestine, according to some authors [35,48•] but not others [49]. Thus, the possibility exists that yet another AEA hydrolysing enzyme may exist in some peripheral organs.

Apart from FAAH, monoacylglycerol lipase (MAGL) activities also inactivate 2-AG (Fig. 2). A MAGL was cloned first from the human and mouse and more recently from the rat. Strong evidence for its role in 2-AG degradation, at least in isolated cells, was provided by showing how 'silencing' of MAGL results in the impairment of 2-AG degradation and in the enhancement of 2-AG, but not AEA, cellular levels [50]. MAGL, however, was found to account for only 50% of the total 2-AG-hydrolysing activity in soluble fractions of rat brain [50], and Stella [51] recently provided pharmacological evidence for the existence of another MAGL enzyme, in agreement with earlier data in macrophages [52]. The cloned MAGL is characterized by poor substrate selectivity: it recognizes as substrates both *sn*-1 and 2-acylglycerols with almost any long chain fatty acid esterified to the glycerol backbone. It is distributed in the CNS in the same brain regions as CB_1 receptors and, unlike FAAH, is a presynaptic enzyme [39], in agreement with the role of 2-AG as a retrograde signal. Our current knowledge of the role of MAGL, however, is still limited due to the lack of a 'knockout' mouse for this enzyme and of selective inhibitors. A series of trifluoromethylketone and methylketone thioether derivatives as inhibitors of 2-AG hydrolysis was reported [53]. Although the authors showed that some of these compounds effectively enhance 2-AG levels in prostate carcinoma cells thereby inhibiting cell proliferation, no data on their selectivity against FAAH or the DAGLs were provided. In another study, the *N*-ethylmaleimide derivative of arachidonic acid (*N*-arachidonylmaleimide) was shown to inhibit 2-AG hydrolysis ($IC_{50} = 180$ nM) [54], again with no data on the selectivity of this compound. Finally, Makara *et al.* [55] recently indicated in URB754 a potent and selective MAGL inhibitor. Subsequent studies, however, have shown that this compound, like its analog URB602, is neither capable of inhibiting 2-AG hydrolysis at concentrations lower than 30 μM nor is it selective against FAAH [56•,57•].

The observation that brain 2-AG levels were not elevated in FAAH null mice or following systemic inhibition of FAAH prompted that this enzyme, despite its capability to recognize 2-AG as substrate, may not be important in the control of 2-AG levels [49]. This conclusion, however, might not be entirely warranted. In fact, it would require the absence or the genetic or pharmacological inactivation of both FAAH and MAGLs, at least, to observe an elevation of 2-AG levels in tissues. Indeed, FAAH inhibitors do produce a significant elevation of 2-AG tissue levels [45,58,59•] if chronically or locally administered, or when 2-AG levels are measured in the intestine [48•]. A cautious interpretation of these data is that both FAAH and MAGLs participate in 2-AG inactivation to an extent depending on the tissue or cell type.

AEA and 2-AG need to be transported across the membrane in order to be released and interact with their binding sites on cannabinoid receptors; and to be inactivated by intracellular enzymes (Fig. 2). A lively debate on whether or not endocannabinoid uptake by, and release from, cells occurs via a plasma membrane transporter, and not via simple passive diffusion, has been ongoing now for over a decade. Intracellular degradation of AEA by FAAH was suggested to be the only drive behind its cellular uptake [60]. Although no specific protein facilitating this process has been identified and cloned, indirect evidence for specific proteins facilitating the membrane transport of both AEA and 2-AG exists and was summarized in recent reviews [61,62]. Recent experiments carried out using cells from FAAH-knockout mice [63,64]; confocal microscopy to assess the spatial and functional separation between anandamide uptake and hydrolysis [65•]; and synthetic compounds, including some carbamoyl tetrazoles, capable of distinguishing between proteins responsible for the binding of AEA to cell membranes and uptake or for its hydrolysis [66,67], have confirmed that FAAH plays a role in, but is not entirely responsible for, AEA cellular uptake. With regard to the carbamoyl tetrazoles, however, it was shown that they are also extremely potent FAAH and MAGL inhibitors [68], thus arguing against their use as tools for the identification of the proteins involved in AEA membrane transport.

Endocannabinoid levels in health and disease

Together with pharmacological studies using THC [1] or selective and efficacious CB_1 and CB_2 receptor agonists and antagonists/inverse agonists, and with observations on the phenotype of CB_1, CB_2 and FAAH 'knock-out' mice, the quantification of anandamide and 2-AG levels in various tissues under physiological and pathological conditions provides important information as to the possible functions of these mediators. In the CNS, endocannabinoids act as retrograde messengers acting on presynaptic CB_1 receptors in both short-term and long-term forms of synaptic plasticity [69]. How these retrograde actions underlie CB_1 regulation of cognitive and

emotional functions in the cortex, hippocampus and amygdala, the reinforcement of substances of abuse in the mesolimbic system, or the control of appetite in the hypothalamus, is being currently investigated [70,71,72•]. CB_1 receptors and endocannabinoids are abundant in the basal ganglia and cerebellum where they control movement and posture, probably also by influencing dopaminergic signalling [73]. The neuromodulatory actions of endocannabinoids in the sensory and autonomic nervous systems result, mostly via CB_1 receptors, in the regulation of circulatory and gastrointestinal functions [74–76] and the hypothalamic–pituitary–adrenal axis [77], whereas direct autacrine or paracrine actions in nonneuronal cells might underlie the several effects of these mediators on female and male reproduction [78], bone formation [79•] and adipocyte and β-cell function [80••]. CB_2 receptors are involved in bone formation and in the humoral immune response, with possible implications in pathological conditions such as (neuro)inflammation and chronic or inflammatory pain [79•,81–83].

How many and what of these functions of the endocannabinoids occur tonically under conditions of physiological homeostasis is at present unclear. The fact that CB_1 and CB_2 receptor knockout, at least in certain genetic backgrounds, does not produce a strong phenotype in unchallenged animals suggests that this system becomes important mostly under pathological conditions. Accordingly, endocannabinoid signalling often undergoes dramatic tissue-specific changes in both animal models of disorders and in human diseases (Table 1) [22•,80••,84•,85•,86,87•,88,90•,91•,92–94,95••,96•,97,119,128•]. In many of these conditions, endocannabinoid levels are controlled through various mechanisms that often differ between AEA and 2-AG, thus reflecting a possible different role for the two mediators (Table 2) [9•,21,23,41,52,98,99,100•,101,102]. There are several examples of regulation of AEA levels via opposite regulation of FAAH activity or expression. Also MAGL is subject to regulation, resulting in corresponding opposing changes in 2-AG levels. There are cases in which the expression or activity of both biosynthetic and degradative enzymes are upregulated, and these changes still lead to upregulation of endocannabinoid levels, such as following induction of neuronal damage with a β-amyloid peptide [22•]. There are also examples of the same stimulus (e.g. leptin) leading to the same change in the tissue concentrations of AEA and 2-AG, but through different regulatory strategies. Finally, as predicted by the pathways shown in Fig. 2, the levels of endocannabinoid precursors are influenced not only by the expression and activity of their biosynthetic and degrading enzymes, but also by the diet and by its relative contents in arachidonic and docosahexaenoic acids. Petersen *et al.* [100•] showed that the amounts of the phospholipid precursors of AEA and 2-AG might be responsible for changes in endocannabinoid levels more than the activity of the direct biosynthesising enzymes.

During some acute pathological states or temporary near-physiological perturbations of the normal homeostasis of the organism, the levels of at least one endocannabinoid in the tissues specifically involved in the disorder are momentarily elevated to help re-establish the normal levels of other endogenous mediators. The subsequent acute activation of cannabinoid receptors occurs, for example, in specific nervous system areas following insults or stressful stimuli, ranging from brief food deprivation, restriction-induced stress and retrieving of aversive memories to administration of an acute painful stimulus, head injury, and ischemic or excitotoxic stimuli [103]. The progressive/chronic nature of some other disorders might result instead in a permanent overactivation of cannabinoid receptors. This, initially, might still help to re-establish homeostasis and exert anti-inflammatory or cell protective effects via both cannabinoid receptor subtypes, but in some cases it also contributes to the development of some of the symptoms typical of the disorder (Table 1). This is the case of experimental models of Parkinson's disease, such as 6-hydroxy-dopamine-treated rats or MPTP-treated marmosets, in which endocannabinoid levels are elevated in the basal ganglia, probably in an attempt to reduce glutamatergic (in the striatum) and GABAergic (in the globus pallidus) signalling, but end up contributing to locomotor impairment via CB_1 receptors [84•,104•]. Interestingly, also the cerebrospinal fluid of Parkinson's disease patients contains elevated levels of the endocannabinoid anandamide [85•]. Likewise, in β-amyloid-treated rats, an animal model of Alzheimer's disease, endocannabinoids protect from microglial cell-driven inflammation [105••] but they also participate in CB_1-mediated loss of memory retention [22•,106]. In animal models of genetic obesity, elevated endocannabinoid levels occur in the hypothalamus and contribute to hyperphagia, or in the adipose tissue, liver and endocrine pancreas, thereby participating in excessive fat accumulation, reduced adiponectin levels and insulin insensitivity that are typical of obesity [80••,94,101,107•,108]. Since higher endocannabinoid levels are also found in the blood and visceral adipose tissue of obese or hyperglycaemic patients [80••,95••,96•] it is likely that the CB_1 antagonist/reverse agonist rimonabant reduces excessive body weight and related metabolic disorders in clinical trials by acting at both central and peripheral levels [109••,110••,111•,112••].

Aberrant endocannabinoid levels and excessive activity at CB_1 receptors might contribute to hepatic fibrosis, a disease typified in humans by overexpression of CB_1 receptors in fibrogenic cells [113••], and hepatic encephalopathy in rats [114], both of which are ameliorated by CB_1 receptor blockade. Osteoporosis was also shown to

Table 1 Changes of endocannabinoid levels and their possible meaning during pathological conditions

Disease	Effects on endocannabinoid levels and possible consequences	References
Neurodegenerative/neuromotor disorders		
Parkinson's disease (PD)	In a nonhuman primate model of PD endocannabinoid levels are elevated in striatum and substantia nigra to compensate for loss of dopamine	[84•]
	Increased levels of *N*-arachidonoyl-ethanolamine (AEA) and decreased levels of 2-arachidonoyl-glycerol (2-AG) in the external globus pallidus may contribute to the generation of parkinsonian symptoms and to expression of levodopa-induced dyskinesia	[84•]
	The cerebrospinal fluid of untreated Parkinson's disease patients contains elevated levels of AEA	[85•]
Alzheimer's disease (AD)	In the hippocampus of β-amyloid-treated rats, an animal model of AD, 2-AG levels are elevated and exert neuroprotection but also participate in memory retention loss	[22•]
Amyotropic lateral sclerosis (ALS)	AEA and 2-AG increase in the spinal cord of SOD1 transgenic mice, a model of ALS	[86,119]
Multiple sclerosis (MS)	In rats with experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, AEA and 2-AG levels are decreased in the striatum and midbrain. This might be associated with motor impairment	[87•,128•]
Epilepsy and neuronal excitotoxicity	AEA levels are elevated in the hippocampus of mice treated with kainic acid. 2-AG levels are elevated in rats treated with pilocarpine. These are two animal models of epileptic seizures, where the endocannabinoids play an anticonvulsant and protective function	[88,89]
Neuropathic pain	Endocannabinoid levels are elevated in periaqueductal grey, rostral ventral medulla and spinal cord during chronic constriction injury of the sciatic nerve in the rat, probably to inhibit pain	[90•]
Gastrointestinal disorders		
Colon inflammation	AEA levels are elevated in the colon of DNBS-treated mice and in the colon submucosa of TNBS-treated rats, two animal models of inflammatory bowel diseases, and in the biopsies of patients with ulcerative colitis, to control inflammation	[91•]
Cholera toxin-induced diarrhoea	Increased AEA levels after administration of cholera toxin to mice, a model of diarrhoea, exert antisecretory action in the small intestine.	[93]
Diverticular disease	Increased AEA levels in colon strips from patients with diverticular disease participate in alterations of neural control of colon motility	[92]
Eating and metabolic disorders		
Anorexia nervosa (AN) and Binge-eating disorder (BED)	Increased blood levels of AEA in patients with AN and BED may participate in reward aspects of the aberrant eating behaviours typical of these disorders	[95••]
Obesity and hyperglycemia	2-AG levels are elevated in mouse adipocytes and epididymal of mice with diet-induced obesity (DIO)	[80••]
	AEA and 2-AG levels are elevated in rat insulinoma β-cells, in pancreas of mice with DIO, and in obese women	[80••]
	Patients with obesity or hyperglycaemia caused by type 2 diabetes exhibit elevated levels of 2-AG or of both endocannabinoids in visceral fat or blood, respectively	[80••,96•]
	AEA levels are elevated in the liver of DIO mice	[94]
Cancer		
Human glioblastoma and meningioma	Elevated levels of AEA in glioblastomas and increased levels of 2-AG in meningiomas might indicate endogenous antitumour actions	[97]

The table summarizes recent data on pathological conditions where endocannabinoid levels have been found to be altered in the tissues or organs affected by the disorder.

be counteracted by CB_1 antagonists [115]. Interestingly, in these three cases where the CB_1 receptor plays the 'bad guy', the CB_2 receptor, often overexpressed, seems to counteract its effects [114,116,117••]. Also in an animal model of amyotropic lateral sclerosis, elevated endocannabinoid levels in the spinal cord induce protective effects that are exerted at least in part via CB_2 receptors [86,118,119]. There are also examples of experimental models of pathological states, where the levels of endocannabinoids need to be neither too high nor too little. Thus, an elegant study suggested that the 'critical balance between anandamide synthesis by NAPE-PLD and its degradation by FAAH in mouse embryos and oviducts creates locally an appropriate 'anandamide tone' for normal development of embryos and their oviductal transport' [120].

In the case of cancer and inflammatory gastrointestinal disorders, both CB_1 and CB_2 might intervene in protective actions exerted by elevated endocannabinoid tissue levels. The higher colonic endocannabinoid levels observed in patients with ulcerative colitis [91•] might cause CB_1-mediated anti-inflammatory and motility inhibitory actions [121]. Enhanced endocannabinoid

Table 2 Mechanisms underlying the regulation of endocannabinoid levels

	Anandamide	2-AG	Refs.
Dietary availability of PUFA precursors	Increased brain levels in piglet and mouse pup brain following increased AA and DHA in milk	Increased and decreased brain levels in mice following decreased or increased DHA in diet, respectively	[98,99]
Availability of NAPE precursors	Increased levels in the rat duodenum after food deprivation		[100•]
Biosynthesis (control exerted by regulating NAPE-PLD and DAGL expression/activity for anandamide and 2-AG, respectively)	In mouse uterus during embryo implantation (stimulated and inhibited)	In microglial cells after P2X7 receptor stimulation (stimulated) In rat brain when passing from the light to the dark phase of the day (inhibited) In rat hypothalamus following systemic administration of leptin (inhibited upstream of DAGL) In the mouse uterus (inhibited by leptin)	[9•,21,23,101,102]
Catabolism (control exerted by regulating FAAH and MAGL expression/activity for anandamide and 2-AG, respectively)	In the mouse uterus (stimulated by leptin); In lymphocytes stimulated with leptin or progesterone (stimulated) In mouse uterus during embryo implantation (stimulated by progesterone)	In macrophages stimulated with LPS (inhibited) In microglial cells after P2X7 receptor stimulation (inhibited);	[23,41,52,102]
Regulation of re-uptake (via the putative membrane transporter)	In the mouse uterus (inhibited by leptin)	No evidence	[23]

AA, arachidonic acid; 2-AG, 2-arachidonoyl glycerol; DHA, docosahexaenoic acid; FAAH, fatty acid amide hydrolase; DAGL, *sn*-1-selective diacylglycerol lipase; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidylethanolamine-selective phospholipase D; LPS, lipopolysaccharide; PUFA; polyunsaturated fatty acid. 'Inhibited' and 'stimulated' refer to 'Biosynthesis' or 'Catabolism' and not to endocannabinoid levels.

signalling was found in some cancer versus noncancer human tissues, or in human tumour cells with an increasingly higher degree of invasiveness [97,122–124] (but see Held-Feindt *et al.* [125] for contrasting data regarding gliomas), and might inhibit cancer cell proliferation or induce apoptosis *in vitro*, and inhibit cancer angiogenesis and metastasis *in vivo* [126•]. Upregulated endocannabinoids might effect their protective function via non-CB_1 non-CB_2 receptors, since treatment with a CB_1 antagonist did not enhance, and in fact reduced, thyroid epithelioma and breast carcinoma cell growth in athymic mice [58,127]. Finally, a more rarely observed reduction of endocannabinoid levels is found in experimental allergic encephalomyelitis, a mouse model of multiple sclerosis [87•,128•,129]. This phenomenon, like in animal models of Huntington's chorea, might explain some of the locomotor impairments typical of the disorder.

Polymorphisms in the genes encoding for endocannabinoid receptors and enzymes, and their association with disease

The studies mentioned in the previous paragraph support the hypothesis that endocannabinoids participate in many pathological conditions, either by protecting from, or by facilitating, the symptoms or progress of diseases. This hypothesis is also supported by the association found between some human pathological states (some of which were described above to be accompanied by changes in endocannabinoid levels) and polymorphisms and genetic variants of the genes encoding for FAAH (*Faah*, see above) as well as of those encoding for the cannabinoid CB_1 and CB_2 receptors (*Cnr1* and *Cnr2*, respectively). The disorders most studied to date include schizophrenia, drug/substance abuse and obesity (for the *Cnr1* and *Faah* genes), neurological and eating disorders

Table 3 Associations between various pathological states and polymorphisms of genes encoding for the CB_1, CB_2 and FAAH proteins

	CB_1 *(Cnr1)*	CB_2 *(Cnr1)*	FAAH *(Faah)*	References
Schizophrenia and psychoses	+/NO	N.I.	NO	[43,130–133]
Substance abuse and dependence	+/NO	N.I.	+	[44,134–137]
Cocaine abuse	+	N.I.	N.I.	[137,138]
Heroin abuse	NO	N.I.	N.I.	[139]
Alcohol abuse and related symptoms	+/NO	N.I.	N.I.	[140,141]
Obesity	N.I.	N.I.	+	[42••]
Osteoporosis	NO	+	N.I.	[142]
Parkinson's disease	+	N.I.	N.I.	[143]
Tourette's syndrome	NO	N.I.	N.I.	[144]
Eating disorders (anorexia and bulimia)	+	N.I.	N.I.	[145]
Autoimmune disorders	N.I.	+	N.I.	[146]

A plus (+) sign indicates the finding of positive correlations, whereas 'NO' denotes the finding of no statistically significant association. Studies of *Cnr1* mostly concern with (AAT)n repeats and single base polymorphisms, whereas the P129T variant is the only polymorphism to have been found to date for *Faah*. In some cases both positive and no correlations have been found depending on the type of mutation examined. N.I., not yet investigated.

(for the *Cnr1* gene only), osteoporosis and autoimmune disorders (for the *Cnr2* gene) (Table 3) [42••,43,44,140–146].

Conclusion

Only the future will tell if drugs manipulating the levels of the endocannabinoids can be developed and administered to human patients for the treatment of disorders in which these mediators are produced and degraded to exert protective actions, or instead contribute to the symptoms. The recent marketing in Europe of rimonabant against obesity and the metabolic syndrome [109••,110••,111•,112••], and of Sativex (GW Pharmaceuticals, Salisbury, UK), a pharmaceutical preparation based on a *Cannabis* extract, against neuropathic pain in multiple sclerosis in Canada [147], suggests that it is possible to interact safely with the endocannabinoid system, and open the way to clinical studies also with inhibitors of endocannabinoid inactivation and biosynthesis, which have been already tested in preclinical studies in disorders ranging from multiple sclerosis, neuronal excitotoxicity, anxiety and depression to chronic pain, hypertension, cancer and gastrointestinal disorders [22•,36,38,58,91•,148–150]. Thus, the future of the endocannabinoid system might be full of surprises more than ever suspected before.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 210–211).

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