Proceedings of the Nutrition Society (2013), **72**, 451–459 doi:10.1017/S0029665113003418
 First published online 11 September 2013

The Nutrition Society Scottish Section Meeting was held at Pollack Halls, Edinburgh on 18–19 March 2013

Conference on 'Polyunsaturated fatty acid mediators: implications for human health' Symposium 3: Cannabinoids in human health

PUFA-derived endocannabinoids: an overview

Maria Grazia Cascio

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK

Following on from the discovery of cannabinoid receptors, of their endogenous agonists (endocannabinoids) and of the biosynthetic and metabolic enzymes of the endocannabinoids, significant progress has been made towards the understanding of the role of the endocannabinoid system in both physiological and pathological conditions. Endocannabinoids are mainly *n*-6 long-chain PUFA (LCPUFA) derivatives that are synthesised by neuronal cells and inactivated via a two-step process that begins with their transport from the extracellular to the intracellular space and culminates in their intracellular degradation by hydrolysis or oxidation. Although the enzymes responsible for the biosynthesis and metabolism of endocannabinoids have been well characterised, the processes involved in their cellular uptake are still a subject of debate. Moreover, little is yet known about the roles of endocannabinoids derived from *n*-3 LCPUFA such as EPA and DHA. Here, I provide an overview of what is currently known about the mechanisms for the biosynthesis and inactivation of endocannabinoids, together with a brief analysis of the involvement of the endocannabinoids in both food intake and obesity. Owing to limited space, recent reviews will be often cited instead of original papers.

Anandamide: 2-arachidonoyl-glycerol: Cannabinoid receptors: Food intake: Obesity

By definition, endocannabinoids are derivatives (amides, esters and ethers) of a long-chain PUFA, specifically arachidonic acid, capable of binding and functionally activating the cannabinoid receptors[1]. It was 1992 when the first endocannabinoid was isolated from brain and named anandamide (from Sanskrit word ananda 'supreme joy')[2,3]. This is the ethanolamide of arachidonic acid, and is thought to be a partial CB_1 and CB_2 receptor agonist as well as a transient receptor potential vanilloid (TRPV)1 receptor agonist[2,4–6]. The other widely investigated endocannabinoid, 2-arachidonoyl-glycerol (2-AG), is the arachidonate ester of glycerol that was isolated from peripheral tissues. This molecule is able to activate CB_1 and CB_2 receptors with similar potency and efficacy[7,8], and to interact with γ-aminobutyric acid receptors[9]. Other endocannabinoids might be represented by both 2-AG ether (noladin ether), that binds to CB_1 receptors with relatively more affinity that to CB_2[10], and virodhamine, that is a CB_2 receptor agonist and CB_1 receptor partial agonist/antagonist[11]. Other compounds that are thought to be endocannabinoids include *N*-arachidonoyl dopamine, that like anandamide behaves as an agonist at both CB_1 and TRPV1 receptors[12] and antagonises the melastatin type 8 (TRPM-8) cation channels[13], *N*-dihomo-γ-linolenoyl ethanolamine and *N*-oleoyl dopamine[14]. Besides the *n*-6 long-chain PUFA, our group recently reported evidence that also the ethanolamides of *n*-3 fatty acids, docosahexaenoyl-ethanolamide (DHEA) and eicosapentaenoyl-ethanolamide, derived mainly from fish oils in the human diet (DHA and EPA) can be classified as endocannabinoids[15]. Indeed, we found that they both are able to bind to CB_1 and CB_2 receptors with reasonable potency and they functionally activate both receptors, although with low efficacy[15]. DHEA was first discovered in brain tissue and retina[16,17].

Abbreviations: ABHD, α/β-hydrolase domain; 2-AG, 2-arachidonoyl-glycerol; COX, cyclooxygenase-2; DAGL, diacylglycerol lipase; DHEA, docosahexaenoyl-ethanolamide; FAAH, fatty acid amide hydrolase; NAPE, *N*-acyl-phosphatidyl-ethanolamine; TRPV, transient receptor potential vanilloid.

Corresponding author: M. G. Cascio, fax +44-1224-437465, email: m.cascio@abdn.ac.uk

In 2001, Berger *et al.*, demonstrated that brain levels of the ethanolamines of DHA and EPA, DHEA and eicosapentaenoyl-ethanolamide (EPEA), in piglets were modulated by the amount of *n*-3 long-chain PUFA in the feed[18]. Other studies have shown an increased formation of DHEA and EPEA in various tissues, including prostate and breast cancer cells, after administering fish oil or individual *n*-3 long-chain PUFA[19,20]. Interestingly, we have also reported evidence that both DHEA and EPEA show greater anti-proliferative effects than their parent compounds, DHA and EPA, in two prostate cancer cell lines, LNCaP and PC3 cells[15]. However, the mechanisms underlying these effects are not clearly understood yet. Furthermore, when released, endocannabinoids are accompanied by cannabinoid receptor-inactive, saturated and mono- or di-unsaturated congeners, which can influence their metabolism and function. They include palmitoylethanolamide, steaeroylethanolamide, oleoylethanolamide, oleamide, 2-linoeoyl-glycerol and 2-palmitoyl-glycerol. These compounds appear to have cannabimimetic activity but do not bind to the classical cannabinoid receptors. It might be possible that these molecules exert their cannabimimetic effects by acting as 'entourage molecules' that prevent anandamide or other true cannabinoids being degraded by specific metabolic enzymes[21]. This hypothesis is supported by the following observations: (a) oleamide greatly increases the efficiency of anandamide binding to cannabinoid receptors[22]; (b) both 2-palmitoyl- and 2-linoleoyl-glycerol have a similar facilitatory effect on 2-AG binding to both cannabinoid receptors as well as on the 2-AG inhibitory effect on forskolin-activated adenylate cyclase[22,23] and (c) these 'entourage' effects were less pronounced in the presence of phenylmethylsulphonyl-fluoride, which inhibits the main metabolic enzyme of anandamide and 2-AG, thus suggesting that these effects were due, at least in part, to inhibition of endocannabinoid hydrolysis by the 'entourage' compounds[22]. Other mechanisms potentially involved in the 'entourage' effects warrant further investigation.

Cannabinoid receptors

Cannabinoid CB_1[24,25] and CB_2[26] receptors belong to the G-protein-coupled receptor superfamily. Their activation inhibits adenylate cyclase and Ca^{2+} (*N*- and P/Q-type) channels, activates K^+ channels and mitogen-activated protein kinase cascades[27], specifically extracellular signal-regulated kinases and p38 mitogen-activated protein kinase cascades[28,29]. Cannabinoid CB_1 receptors are mainly expressed in the central nervous system where they mediate inhibition of ongoing release of various neurotransmitters (acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine, γ-aminobutyric acid, glutamate, D-aspartate and cholecystokinin)[30,31], and at lower levels in testis, heart, vascular tissue and in immune cells. Within the central nervous system, CB_1 receptors are highly expressed in the cerebral cortex, hippocampus, lateral caudate-putamen, substantia nigra pars reticulate, globus pallidus, entopeduncular nucleus and cerebellum as well as in the pain pathways in brain and spinal cord. In these areas endocannabinoids control processes such as cognition, memory, motor function and analgesia[32]. Unlike CB_2, CB_1 receptors are associated with special membrane microdomains, named 'lipid rafts'[33]. This association is greatly affected by cholesterol content; indeed, membrane cholesterol enrichment in both primary and immortalised cell lines reduces the binding to CB_1; instead cholesterol depletion modifies anandamide-induced endocytosis of CB_1, which apparently loses the ability to be directed towards the lysosomal compartment[33]. Importantly, the existence on the CB_1 cannabinoid receptors of an allosteric binding site that can be recognised by synthetic small molecules was reported for the first time by our group[34]. Whether the CB_2 receptor, such as CB_1, possesses an allosteric binding site, warrants further investigation. Cannabinoid CB_2 receptors are mainly expressed in immune cells, and recently they have also been detected in microglia, astrocytes and in central neurons[35,36]. Finally, the existence of a third type of cannabinoid receptor, GPR55, is still a subject of debate[37].

Endocannabinoids biosynthesis and uptake

Although the biosynthetic and metabolic pathways have been largely studied for the *n*-6 endocannabinoids, it is probably that similar routes can occur for the *n*-3 endocannabinoids. Endocannabinoids are not stored in cells such as classical neurotransmitters waiting to be released after cell stimulation, but instead they are rapidly formed from membrane phospholipids 'on demand', where and when needed, and immediately released to target cannabinoid receptors mainly locally. Although anandamide and 2-AG are similar in structure, these endocannabinoids exhibit some differences in terms of biochemical and metabolic pathways. Both endocannabinoids are produced at post-synaptic neurons. For anandamide, the main biosynthetic pathway consists of a two-step process: (1) formation of *N*-acyl-phosphatidyl-ethanolamine (NAPE) from phosphatidyl-ethanolamine by a calcium-dependent *N*-acyltransferase, and (2) hydrolysis of NAPE to form N-acylethanolamines in a process that is catalysed by NAPE-hydrolysing phospholipase D[38–40] (Fig. 1). Since cells lacking NAPE-phospholipase D are also able to synthesise anandamide, alternative pathways have been proposed[41–47] and they are summarised in Fig. 1. The main biosynthetic pathway for 2-AG consists of hydrolysis by phospholipase C of inositol phospholipids containing arachidonic acid at the *sn*-2 position and further hydrolysis by diacylglycerol lipase (DAGL) of the arachidonic acid-containing DAG to 2-AG[48] (Fig. 2). In 2003, human DAGL was cloned and further characterised[49]. It exists as two closely related genes designated α and β[49]. Pharmacological studies have revealed that during neuronal development, localisation of DAGLα and DAGLβ changes from pre- to post-synaptic elements, i.e. from axonal tracts in the embryo to dendritic fields in the

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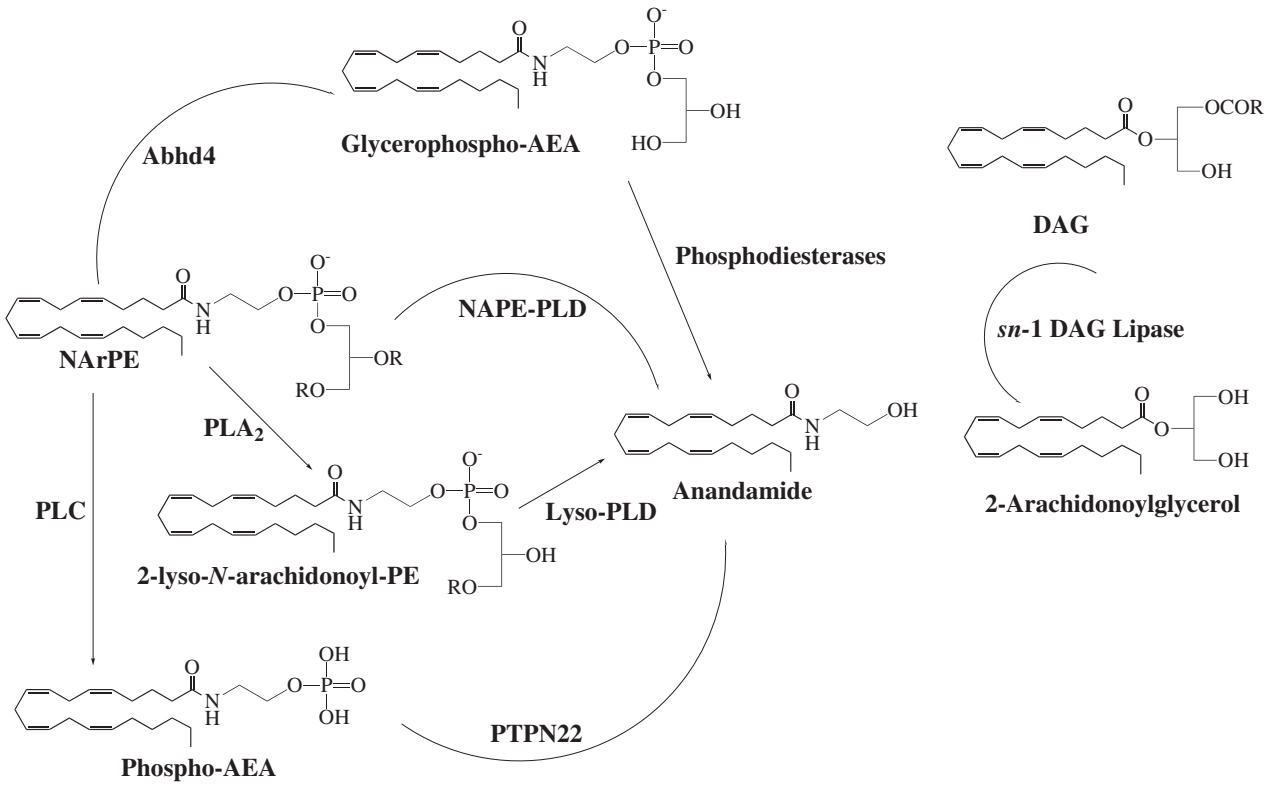

Fig. 1. Schematic representation of anandamide and 2-arachidonoyl-glycerol biosynthesis routes. AEA, anandamide; NAPE, N-acylphosphatidyl-ethanolamine; NArPE, *N*-arachidonoylphosphatidylethanolamine; PLC, phospholipase C; PTPN22, protein tyrosine phosphatase; PLA_2, phospholipase A_2; PE, phosphatidyl-ethanolamine; PLD, phospholipase D; Abhd4, α/β-hydrolase 4; DAG, diacylglycerol.

adult, suggesting a different need for 2-AG synthesis from pre- to the post-synaptic compartment during brain development[49,50]. Furthermore, several studies suggest that DAGLα plays an essential role in the regulation of retrograde synaptic plasticity and neurogenesis. In support of this hypothesis two recent studies suggest that: (1) DAGLα-knockout mice show marked (up to 80%) reductions in 2-AG levels in brain and spinal cord with concomitant decrease in arachidonic acid levels, whereas DAGLβ-knockout animals exhibited either no[51] or up to 50% reduction[52] in brain 2-AG levels; (2) several forms of retrograde endocannabinoid-mediated synaptic suppression, such as depolarisation-induced suppression of excitation and depolarisation-induced suppression of inhibition, were absent in hippocampus, cerebellum and striatum in DAGLα-knockout, but not in DAGLβ-knockout mice[51–53]. Like anandamide, also 2-AG can be synthesised by alternative pathways. However, the physiological meaning of these proposed pathways is not yet clear. Endocannabinoids function as retrograde messengers. Indeed, after their biosynthesis, they are released from post-synaptic neurons upon post-synaptic depolarisation and/or receptor activation and act on presynaptic CB_1 receptors to induce transient suppression of transmitter release. Two forms of short-term synaptic plasticity have been identified so far, named depolarisation-induced suppression of inhibition, which involves GABAergic transmission, and depolarisation-induced suppression of excitation, which involves glutamatergic transmission[54,55]. These processes were found mainly in the hippocampus and cerebellum, where it seems they play an important role in physiological processes such as memory and motor co-ordination[56–58]. Additional forms of synaptic transmission involve the induction of long-term synaptic plasticity, named long-term potentiation and long-term depression[59]. After targeting their receptors, the endocannabinoids are inactivated by a two-step process. The first process is the endocannabinoid transport from the extracellular to the intracellular space, followed by their metabolism. Whether this cellular uptake depends on the presence of an 'endocannabinoid membrane transporter' is currently a subject for debate as no such transporter has yet been cloned. Recently, Fowler has elegantly reviewed the current state of the art of endocannabinoid uptake[60].

Endocannabinoid metabolism

Whatever is the mechanism by which endocannabinoids are taken up by cells, after the uptake, they are metabolised by hydrolysis or oxidation (Fig. 2).

Hydrolysis

Fatty acid amide hydrolase (FAAH) is the main enzyme involved in anandamide hydrolysis, and it is able to recognise as substrates also other *N*-acyl-ethanolamines

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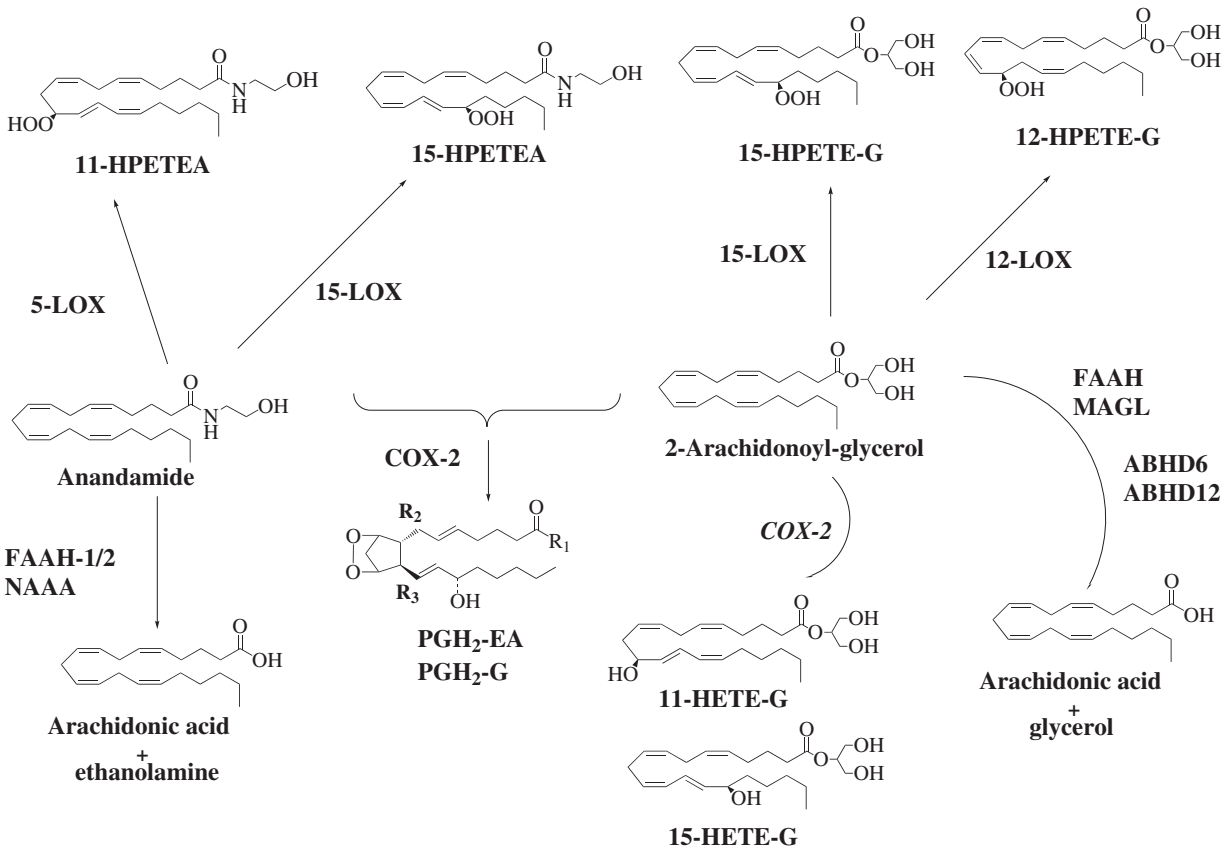

Fig. 2. Schematic representation of anandamide and 2-arachidonoyl-glycerol metabolic routes. HETE, hydroxyeicosatetraenoic acid; HPETEA, hydroxyperoxyeicosatetraenoylethanolamide; LOX, lypoxygenase; COX, cyclooxygenase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAAA, *N*-acylethanolamine-hydrolysing acid amidase; ABHD, α/β-hydrolase domain.

such as oleamide[61,62], and *N*-acyl-taurines[63]. FAAH is a membrane-associated serine hydrolase belonging to the amidase signature family[64]. The catalytic triad is composed of Lys^{142}, Ser^{217} and Ser^{241}[65]. This enzyme is widely distributed in various tissues of rat[64,66,67], mouse[68,69] and human subjects[61,70], and its optimal pH lies within the range 8·5–10. Other enzymes involved in anandamide hydrolysis are N-acylethanolamine acid amidase[71] and FAAH-2[70], this latter being an isozyme of FAAH-1 with about 20% sequence identity at the amino acid level, mainly expressed in human subjects, but not in rodents[70]. N-acylethanolamine acid amidase is an *N*-glycosylated protein, localised in the lysosomes or the Golgi apparatus with an optimal pH of 4·5–5[71–74]. FAAH-2 is more effective at metabolizing oleamide than anandamide or other *N*-acyl-ethanolamines. FAAH-1 and FAAH-2 are located in the cytosolic and luminal sides of intracellular membranes, respectively. FAAH is also able to metabolise, although to a lesser extent, 2-AG[75,76]. Recently, three 'guardians' of 2-AG signalling have been reported: monoacylglygerol lipase, α/β-hydrolase domain (ABHD)-6 and ABHD-12. As recently reviewed[53], MAG lipase is a serine hydrolase belonging to the α/β-hydrolase superfamily, whose catalytic triad is composed of Ser^{122}, Asp^{239} and His^{269}[77,78]. It was originally purified, and subsequently cloned from adipose tissue[77,79], and it is detected in both cytosol and membrane preparation[80]. This enzyme accounts for about 80–85% of 2-AG hydrolysis, but it is also involved in the hydrolysis of other 2-monoacylglygerols and 1-monoacylglygerols. Its localisation is mainly presynaptic, where it often co-localises with CB_1 receptors in the axon terminals[81]. ABHD-6 and ABHD-12 belong to the α/β-hydrolase family with the postulated catalytic triad serine-aspartic acid-histidine[53,82]. ABHD-6 is mainly post-synaptic where it regulates 2-AG levels at the site of its generation[83]. This enzyme is also expressed in the mouse microglial cell line BV-2, in which monoacylglygerol lipase is not expressed[83]. The ABHD-6 catalytic triad has not been resolved yet, but it is predicted to face the cytosol/intracellular membrane[53,75]. ABHD-12 is highly expressed in microglia, macrophages and osteoclasts. Its catalytic triad is not resolved yet but it is predicted to face the luminal/extracellular side[53,75]. Inactivating ABHD-12 mutations have been causally linked to neurodegenerative conditions, known as polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract[53,84].

Oxidation

Both endocannabinoids can also be metabolised by oxidation involving enzymes such as cytochrome P-450, cyclooxygenase-2 (COX-2) and by the 12- and

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15-lipoxygenases, 12-LOX and 15-LOX[85] (Fig. 2). Specifically, anandamide can undergo oxidation by several human cytochrome P-450 isoenzymes, including CYP3A4, CYP4F2, CYP4X1 and the polymorphic CYP2D6 resulting in a number of structurally diverse epoxy derivatives that are still able to act on both cannabinoid receptors, CB_1 and CB_2, and on vanilloid TRPV4 receptors[86]. Little evidence exists for the oxidation of 2-AG by any P-450 enzymes from different tissue preparations[21]. Lipoxygenases generate hydroxyl-endocannabinoids that activate both cannabinoid receptors and vanilloid TRPV1 receptors[86]. Finally, Yu *et al.*[87] showed that COX-2 but not COX-1 oxygenates anandamide, indicating substrate specificity for the two isoforms. The catalysis mediated by COX-2 induces the formation of both prostamides and PG-glycerol esters, that do not appear to act as ligands for CB_1 and CB_2 cannabinoid receptors or of any of the EP1-4 eicosanoid receptors, but that have been shown to act through other receptors such as PPAR and NF-κB receptors[21].

The physiological and pathological roles of the endocannabinoids

Under physiological conditions, the endocannabinoid system has been reported to modulate several other systems that range from the central and autonomic nervous systems to the endocrine system, the gastrointestinal tract and the reproductive, immune and cardiovascular systems[14,88]. Furthermore, there is convincing evidence too that the endocannabinoid system plays a crucial role also in several pathological conditions. An up-regulation of the endocannabinoid system has already been observed in a wide range of disorders such as multiple sclerosis, cancer, schizophrenia, post-traumatic stress disorders, certain types of pain, some intestinal and cardiovascular diseases, excitotoxicity and traumatic head injury. On the one hand, this up-regulation may play an 'autoprotective' role with consequent reduction of the severity of symptoms or a slowing of disease progression[14,27]. However, there are also disorders in which up-regulation of the endocannabinoid system contributes to the production or exacerbation of unwanted effects, and so is 'auto-impairing'[14,27]. These disorders include obesity, impaired fertility, stroke, cystitis, ileitis and paralytic ileus. It will be really important to understand which are the mechanisms underlying the alterations in the endocannabinoid levels and to explore whether these are due to an increase in their biosynthesis or a decrease in their enzymatic degradation. Here, I will briefly describe the role played by the endocannabinoid system in the control of food intake (physiological role) and in obesity (pathological role).

Endocannabinoids in the control of food intake and energy expenditure

In mammals, the need for feeding is governed by endogenous controllers that include signals released from the gastrointestinal tract after meals, such as ghrelin, cholecystokinin, and peptide YY, as well as signals more strictly related to metabolism, such as the circulating hormones insulin and leptin[19]. All stimuli involved in feeding are centrally integrated by the hypothalamus, a brain area known to play an important role in homoeostatic control, thus maintaining an adequate body weight[19]. There is now convincing evidence that the endocannabinoid system is involved in the regulation of food intake and energy expenditure. This is supported by the following observations: (i) Δ^9-tetrahydrocannabinol, the main psychotropic ingredient of *Cannabis sativa*, has been found to induce signs of hyperphagia by activating cannabinoid CB_1 receptors[89,90]. Indeed, tetrahydrocannabinol was found to improve appetite and increase body weight in advanced cancer patients or in anorexic patients with AIDS or Alzheimer's disease[91]. Moreover, (ii) cannabinoid CB_1 receptors are activated after brief food deprivation in a manner that increases the levels of orexigenic and anorexigenic mediators and induces food intake[92]; (iii) the levels of endocannabinoids in the hypothalamus are higher in rodents deprived of food for several hours *v. ad libitum*-fed animals[92]; and (iv) when directly injected into the hypothalamus or the nucleus accumbens shell, endocannabinoids induce food intake in satiated animals[92]. The fact that all these effects are attenuated by CB_1 receptor antagonists strongly supports a role of the endocannabinoid system in the regulation of food intake. Accordingly, cannabinoid CB_1 receptors have been found to exert both central and peripheral effects on food intake and energy homoeostasis[93]. In the central nervous system, cannabinoid CB_1 receptors have been found in the olfactory bulb, cortical regions (neocortex, pyriform cortex, hippocampus and amygdala) and several parts of the basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex, brainstem nuclei as well as in areas involved in reward/reinforcement circuitry[93]. Furthermore, cannabinoid CB_1 receptors have been found to co-localise with other receptors in the central nervous system whose activities are essential in the processes of feeding and satiety. For example, the dopaminergic system, which is involved in reward regulation, interacts with CB_1 receptors and co-localisation between dopamine receptors (D_1 and D_2) and CB_1 receptors was reported in mouse hippocampus (CB_1 and D_2), and striatum and olfactory tubercle (CB_1, D_1 and D_2)[93]. In addition, it has been found that cannabinoid CB_1 receptor antagonists, such as SR141716 (also known as rimonabant), AM251 or AM1387, suppress food intake and disrupt food-reinforced behaviour[94]; that food-deprived $CB_1^{-/-}$ mice eat less than their wild-type littermates (SR141716 does not affect the food intake of these animals)[95,96] and that levels of endocannabinoids are elevated in leptin-deficient mice and rats, suggesting that endocannabinoids form part of the leptin-regulated neural circuitry that is involved in appetite regulation[95]. In periphery, the endocannabinoid system acts directly to regulate processes such as gastric emptying, lipogenesis and glucose intake[97] through cannabinoid

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receptors expressed by peripheral cells and tissues controlling energy homoeostasis, including the gut, the liver and hepatocytes, white adipose tissue, and adipocytes, skeletal muscle and the pancreas[92]. In this way, signals from these peripheral organs can be collectively converged and fed back centrally, allowing the brain to constantly monitor the metabolic state of an organism[93].

Endocannabinoids in obesity

Besides its role in the regulation of food intake, there is also evidence that the endocannabinoid system is overactivated and dysregulated in human obesity. Obesity is a pathological condition whose incidence continues to increase as a global nutrition and health problem. One of the key factors leading to obesity is a significant imbalance between energy intake and expenditure. In addition, the high amount of *n*-6 PUFA, such as linolenic acid and arachidonic acid, over the *n*-3 PUFA, in the Western diet has hugely contributed to the onset of obesity. Unfortunately, the mechanisms by which different fatty acids contribute to obesity are not well-understood yet and further research is needed. The involvement of the endocannabinoid system in obesity is supported by the following observations: (i) CB_1 receptor antagonists are significantly more efficacious in reducing caloric intake and body weight in rodents with diet-induced or genetic obesity than in their respective lean controls[95,98,99]; (ii) $CB_1^{-/-}$ mice are resistant to diet-induced obesity[100,101]; and (iii) both an up-regulation of CB_1 receptors and elevated endocannabinoid levels have been detected in the adipose tissue of obese compared with lean patients[102,103]. Importantly, CB_1 receptor antagonists show significant anti-obesity effects. Rimonabant, which is a CB_1 receptor inverse agonist/antagonist, has been found (i) to reduce food intake in both lean and obese rodents and to lower body weight both in experimental models of obesity and in clinical trials[104]; (ii) to decrease fat intake as well as hunger ratings[104]; and (iii) to improve waist circumference, plasma TAG, HDL cholesterol and blood pressure[104]. Rimonabant was approved in 2006 as a weight loss medication in the European Union. Unfortunately, however, the use of this drug in the clinic has been suspended because of serious psychiatric side effects, particularly an increased incidence of depression and suicidality.

In this regard, the use of CB_1 receptor antagonists that do not cross the blood–brain barrier might provide a novel pharmacological approach to controlling obesity without the psychiatric side-effects observed with rimonabant and its analogues. In addition, the development of 'neutral' CB_1 antagonists, that do not show any significant signs of inverse agonism, has provided very promising results at the preclinical level, particularly in terms of their reversal of insulin and leptin resistance[105]. Furthermore, in the light of the fact that the increased endocannabinoid tone observed in metabolic disorders can be attributed to increased endocannabinoid biosynthesis, an alternative strategy to regulate dysregulated endocannabinoid tone in obesity might be to use DAGL inhibitors with consequent reduction in 2-AG biosynthesis[106]. Finally, changes in diet can be beneficial in preventing the onset of both obesity and other metabolic disorders. Indeed, several data reported in the literature suggest that dietary intake can modulate the endocannabinoid system. Thus, high-fat diets increase intestinal motility and the levels of the endocannabinoids, probably due to decreased monoacylglygerol lipase and FAAH activity and increased NAPE-phospholipase D action[107]. Interestingly, the role of dietary fish oil *n*-3 fatty acids, EPA and DHA, in modulating endocannabinoid biosynthesis has been widely studied. Indeed, increased intake of EPA and DHA, that are able to displace arachidonic acid from phospholipid membranes, not only contributes to a marked decrease in endocannabinoid biosynthesis, but also causes a decrease in NAPE-phospholipase D, FAAH and CB_1 mRNA expression with a consequent reduction of receptor stimulation[107]. However, such a change in diet should be considered with caution in newborn since it can cause long-lasting alterations in brain phospholipid composition and function[105].

Conclusions and future directions

It is now generally accepted that the endocannabinoid system plays a crucial role in several physiological processes and pathological conditions in both central and peripheral tissues. One challenge now is to develop: (a) new peripherally restricted CB_1 receptor agonists and/or antagonists that while maintaining the sought-after therapeutic effect do not show the unwanted side-effects that have been observed with direct CB_1 ligands which cross the blood–brain barrier; (b) new medicines that affect the tissue level of endocannabinoids at their receptors for the treatment of a range of disorders, such as, to mention just a few, pain, multiple sclerosis, hypertension and cancer.

Acknowledgements

I wish to thank Professor Roger G. Pertwee (University of Aberdeen) for his continuing support and advice.

Financial Support

This was provided by GW Pharmaceuticals and Otsuka. No one from GW Pharmaceuticals or Otsuka had a role in the design, analysis or writing of this article.

Conflicts of Interest

None.

https://doi.org/10.1017/S0029665113003418 Published online by Cambridge University Press

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