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Review

# 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance

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**Abstract**

The only endogenous substances isolated and characterised so far that are capable of mimicking the pharmacological actions of the active principle of marijuana, (−)-$\Delta^9$-tetrahydrocannabinol, are amides and esters of fatty acids. Some of these compounds, like anandamide (*N*-arachidonoylethanolamine) and 2-arachidonoylglycerol, act as true 'endogenous cannabinoids' by binding and functionally activating one or both cannabinoid receptor subtypes present on nervous and peripheral cell membranes. The metabolic pathways and molecular mode of actions of these metabolites, as well as their possible implication in physiopathological responses, are reviewed here. © 1998 Elsevier Science B.V.

*Keywords:* Cannabinoid; Anandamide; Arachidonic acid; Eicosanoid; Oleamide; 2-Arachidonoyl-glycerol

## Contents



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## 1. Introduction

Opium and cannabis are probably the two oldest plant-derived recreational and therapeutic drugs of abuse in human history. Still, both the active principle of opium and its possible molecular mechanism of action were discovered several decades before the corresponding findings could be made for marijuana and hashish, the two most popular preparations from *Cannabis sativa*. The somewhat slower development of cannabis research compared to opiate research is not due to a lack of interest from the scientific community towards cannabinoid pharmacology. Indeed, the therapeutic exploitation of Indian hemp, which included the treatment of cramps, migraine, convulsions, neuralgia, nausea, diarrhoea, asthma and anorexia, had been already recognised by several Western physicians in the early nineteenth century [1]. It was probably the lipophilic nature of the most abundant active principle of cannabis, (−)-$\Delta^9$-tetrahydrocannabinol (THC), that made its structure elucidation and total chemical synthesis difficult (these were obtained, respectively, only in 1964 and 1981 [2,3]), and somehow delayed its pharmacological characterisation. The latter revealed that THC was responsible for most psychotropic effects of marijuana smoking (for a review see [4]). It was again THC's lipophilic nature, together with the difficulties encountered in preparing a stereochemically pure preparation of this or related compounds, that suggested a mechanism of action for psychoactive 'cannabinoids' similar to that of cell membrane perturbing anesthetics [5], thus delaying the finding of stereoselective cannabinoid binding sites until 1988 [6]. As with morphine, the discovery of receptors for *xenobiotic* cannabinoids raised the possibility of the existence in mammalian tissues of *endogenous* counterparts. This possibility found conclusive experimental evidence only in 1992 [7] with the finding of *anandamide*, the amide of arachidonic acid with ethanolamine, which took its name from the Sanskrit word for bliss, *ananda.* Since the isolation of anandamide, several other long-chain fatty acid amides and esters, including the ethanolamides of palmitic, di-homo-γ-linolenic and docosatetraenoic acids, *cis*-9-octadecenoamide and 2-arachidonoylglycerol, have been found to exhibit some cannabimimetic properties in vitro and/or in vivo. The latest developments of studies on the molecular mechanisms underlying the pharmacological action, biosynthesis and inactivation of these compounds as well as their possible involvement in physiopathological responses are reviewed in this article.

## 2. Cannabimimetic metabolites, anandamides and 'endocannabinoids'

A recent definition of cannabimimetic activity as the whole of cannabinoid receptor-mediated pharmacological properties exhibited by the prototypical cannabinoid THC was given by Pertwee [8]. This

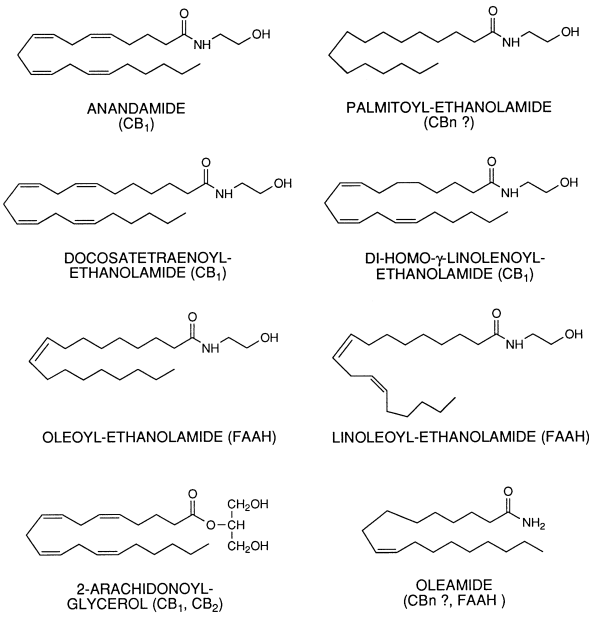


Fig. 1. Chemical structures of 'endocannabinoids' and other fatty acid derivatives with cannabimimetic properties. The putative targets mediating the cannabimimetic actions of the metabolites are shown in parentheses. CB1, cannabinoid receptor subtype 1; CB2, cannabinoid receptor subtype 2; CBn, unknown cannabinoid receptor subtype; FAAH, fatty acid amide hydrolase.

definition, however, does not include the several non-cannabinoid receptor-mediated actions so far described for THC (for a review see [9]). Hence, a more literal and wider, but nonetheless specific, definition of cannabimimetic activity as the capability of mimicking THC actions both in vivo and in vitro, whether they are mediated by cannabinoid receptors or not, can be proposed. According to this definition, a compound should be indicated as *cannabimimetic* as long as it proves to be 'THC-mimetic' in the long series of bioassays described in the literature (and reviewed in [10]) where the cannabinoid compound has been found to be active. A preliminary way of assessing cannabimimetic activity was suggested by Martin et al. in 1991 [11], who measured the effect of putative cannabimimetic compounds using a 'tetrad' of mouse behavioural assays that, when performed together, are highly indicative of cannabinoid-like activity. These assays are: (a) the 'open-field' test, which measures locomotor activity, (b) the 'ring immobility' test, where the time spent motionless in a ring is measured, (c) the 'hot plate' test, used to determine antinociceptive effects, and (d) induction of rectal hypothermia. Even though a positive response in any of these assays is not specific for cannabinoids, only cannabinoid-like compounds have so far been found to exhibit activity in all four tests in the same range of concentrations [11]. Cannabimimetic activity can then be further assessed by measuring cross-tolerance to THC in these assays, or by determining whether or not animals can distinguish between the effects of the compound under study and those of THC (drug discrimination test). In the former case, a positive response is highly suggestive of a cannabinoid receptor-mediated action. This can then be tested in vitro by using receptor binding competition assays carried out with membrane preparations from cells overexpressing cannabinoid receptors.

The discovery of anandamide (*N*-arachidonoyl-ethanolamine) in pig brain [7], and the finding that this compound, apart from being cannabimimetic in the 'tetrad' of tests [12–14] and inhibiting the electrically stimulated mouse vas deferens twitch response (another typical, albeit unselective, effect of THC), could selectively bind to and functionally activate cannabinoid receptors, confirmed the hypothesis of the existence of endogenous ligands for such receptors. Although structurally different from plant cannabinoids, these metabolites, in analogy with the 'endorphins', i.e. the endogenous ligands of opiate receptors, can be named '*endocannabinoids*' [15]. Thus, 'endocannabinoids' can be defined as endogenous cannabimimetic substances capable of functionally activating one or both cannabinoid receptor subtypes characterised so far in animals (see Section 3). Two natural anandamide congeners, *N*-di-homo-γ-linolenoyl- and *N*-docosatetraenoyl-ethanolamines, were also isolated from pig brain [16], and found to exert cannabimimetic actions [17,18] as well as binding to and functionally activating cannabinoid receptors [16]. It was proposed that the three 'endocannabinoid' *N*-acylethanolamines (NAEs) be named *anandamides*, to distinguish them from non-'endocannabinoid' NAEs, whose metabolism and action had already been investigated in the 1970s and 1980s (for a review see [19]). One member of this family of lipids, *N*-palmitoylethanolamine, was found to share with cannabinoids an anti-inflammatory action in mast cells and basophils [20] as well as a neuroprotective effect in cerebellar granule cells

[21]. However, since it does not bind with high affinity to either of the two cannabinoid receptor subtypes [22,23], *N*-palmitoylethanolamine cannot be defined as an 'endocannabinoid' nor as an anandamide, but rather as a substance with cannabimimetic actions in some assays. The same applies to *cis*-9-octadecenoamide (*oleamide*), a substance isolated from the cerebrospinal fluid of sleep-deprived mammals and shown to induce normal sleep in rats [24]. Despite being incapable of binding to either cannabinoid receptor subtype [25], oleamide shares with THC and anandamide a significant, albeit weak, activity in the 'tetrad' of mouse assays [26], as well as an immunosuppressive action on lymphocytes in vitro [27]. Finally, another arachidonic acid derivative, 2-arachidonoylglycerol, found in both canine gut [28] and rat brain [29], was shown to mimic THC in all pharmacological models tested so far [28,30], and to functionally activate cannabinoid receptors [28]. This compound, according to the definitions suggested above, should be considered an 'endocannabinoid', but not an anandamide.

Thus, not all endogenous metabolites that exhibit some cannabimimetic properties are 'endocannabinoids', and not all 'endocannabinoids' are anandamides. Moreover, 'endocannabinoids' may not mimic some of the non-cannabinoid receptor-mediated actions of THC and, therefore, may not be cannabimimetic in some assays. Although originally proposed to refer to 'endocannabinoid' NAEs [16], the term anandamide is currently used only for the most thoroughly studied member of this family, i.e. *N*-arachidonoylethanolamine. Other cannabimimetic fatty acid amides, whose possible molecular target is not represented by cannabinoid receptors (Fig. 1), have been isolated from both mammalian and invertebrate tissues, and will be also discussed in this article.

## 3. 'Endocannabinoid' molecular targets

### 3.1. Cannabinoid receptor-mediated actions

Two cannabinoid receptor subtypes, termed CB1 and CB2, have been characterised and cloned to date [31,32]. Both receptors belong to the family of the 'seven *trans*-membrane spanning receptors', and their functional response is mediated by pertussis toxin-sensitive GTP-binding ($G_{i/o}$) proteins. The CB1 receptor, previously known as the 'central' cannabinoid receptor, is widely distributed in several brain regions [33], with the highest density in the cortex, hippocampus, basal ganglia and cerebellum, as well as in the cervical ganglion and peripheral autonomic fibres, such as those innervating the vas deferens, urinary bladder and heart (reviewed in [8]). CB1 is also present in non-nervous tissues such as the prostate, uterus, testes, small intestine, spleen and lymphocytes [8]. A CB1 receptor variant, named CB1a, has also been described [34]. The CB2 receptor seems to be confined to immune tissues, such as the spleen, thymus and tonsils, and immunocompetent blood cells, with the highest concentration in B-cells, natural killer cells, mast cells and monocytes [8]. Anandamide and 2-arachidonoylglycerol have been shown to bind to both CB1 and CB2 receptors and to elicit a typical CB1 and CB2 functional response, i.e. the $G_{i/o}$-mediated inhibition of forskolin-induced cAMP formation. This could be observed either in CHO and COS-7 cells transfected with CB1/CB2 receptor cDNA [28,30,35,36], or in cell lines or tissue preparations constitutively expressing either receptor subtype [8]. Interestingly, CB1, but not CB2, receptors have been recently suggested to also activate adenylate cyclase via a $G_s$ protein, an effect that is usually masked by the more typical $G_i$-mediated inhibition of the enzyme, and which could only be observed after treating the cells with pertussis toxin [37]. This effect may explain why anandamide – which also stimulates forskolin-induced cAMP formation in pertussis toxin-treated CHO cells overexpressing CB1 receptors [199] – at low doses exhibits 'excitatory' rather than 'inhibitory' actions in several behavioural tests, and inhibits some THC-induced pharmacological actions [38,39,200]. Apart from adenylate cyclase, CB1 receptors are coupled, again through G-proteins, to inhibition of N-type and P/Q-type $Ca^{2+}$ channels, and, accordingly, anandamide was found to inhibit $Ca^{2+}$ influx through these channels in N18TG2 mouse neuroblastoma cells, CB1-transfected AtT-20 cells and rat hippocampal neurones [40–43]. Moreover, anandamide, by acting at CB1 receptors, also activates an inwardly rectifying $K^+$ current in transfected AtT-20 cells [41,42]. Surprisingly, low nM concentrations of 2-arachidonoylglycerol, by acting through CB1 receptors and phos-

pholipase C activation, were recently shown to induce a transient increase in the intracellular $Ca^{2+}$ concentration in undifferentiated N18TG2 and NG108x15 cells [43], whereas higher doses exhibited a more typical inhibition of $Ca^{2+}$ influx in differentiated cells [44]. The former action could be observed with anandamide only at higher concentrations and to a lower extent. Both CB1 and CB2 receptors were found to be coupled also to mitogen-activated protein kinase (MAPK) independently of adenylate cyclase inhibition [45,46], but the only report of a pertussis toxin-sensitive stimulatory effect of anandamide on MAPK was obtained in WI-38 human lung fibroblasts [47], where no data for the presence of either cannabinoid receptor subtype are available. CB2-mediated MAPK activation was found to induce *krox-24* gene expression in HL60 cells [46], but again no such effect for anandamide or 2-arachidonoylglycerol has been reported yet. Cannabinoids and anandamide were shown to activate a neuronal form of focal adhesion kinase (FAK+) in rat hippocampal slices in a fashion sensitive to the CB1 receptor-selective antagonist SR 141716A [48], as well as to pertussis toxin and PKA inhibitors or activators [49], thus suggesting that the 'endogenous cannabinoid system' (ECS) is coupled to FAK+ via CB1 receptors and adenylate cyclase inhibition. Cannabinoids and anandamide exert a dual action on NO synthases in both nervous and non-nervous tissue, either by stimulating basal NO release in *Mytilus* and leech ganglia, *Mytilus* immunocytes and rat renal endothelial cells [50–52,71], or by inhibiting lipopolysaccharide-induced NO formation in macrophages [53]. This dual action may reflect the participation of either CB1 or CB2 receptors as well as their capability to couple to either constitutive or inducible NO synthase. Finally, the psychoactive synthetic cannabinoid HU-210, anandamide and 2-arachidonoylglycerol were recently shown to potently inhibit human breast cancer cell proliferation through CB1-mediated suppression of prolactin receptor levels [54].

### 3.2. CBn (non-CB1/non-CB2) cannabinoid receptor-mediated actions

The discovery of the 'endocannabinoids' indirectly led to the suggestion that non-CB1/non-CB2 (CBn) cannabinoid receptor subtypes exist in both the CNS and periphery. THC and anandamide, in the low–medium μM range of concentrations, induce arachidonic acid release via phospholipase $A_2$ activation in both central and peripheral cell types [47,55–57]. This effect is blocked by high concentrations of SR 141716A in astrocytes [55], by adenylate cyclase-activating agents and/or pertussis toxin in lung fibroblasts and J774 mouse macrophages [47,56], and by either SR 141716A or CB1 and CB2 antisense oligonucleotides in N18TG2 cells and RAW 264.7 macrophages [57]. In J774 cells the effect appeared to be biphasic with a first plateau being reached at 50 μM and a further increase at higher concentrations [56]. Indeed, anandamide, in the medium–high μM range, induces arachidonate liberation also in cells that do not express CB1/CB2 receptors [36,42]. It is possible that a CBn receptor, with low affinity for anandamide and high homology with CB1/CB2 receptors, is coupled to phospholipase $A_2$ activation via adenylate cyclase inhibition and/or $G_i$ proteins. The presence of CBn receptors in astrocytes was also suggested by the finding of an inhibitory action by anandamide on gap junction-mediated and glutamate-triggered $Ca^{2+}$ waves [58]. This effect was sensitive to pertussis toxin but was unaffected by SR 141716A, and so was also the anandamide-induced depletion of $Ca^{2+}$ from fast mobilisable, glutamate-activated, internal stores in the same cells [59]. A putative astrocyte CBn receptor might also accommodate oleamide, reported to share with anandamide its inhibitory action on astrocyte gap junctions [190]. Finally, the existence of CBn receptors may be suggested also from data obtained with *N*-palmitoylethanolamine which was found to displace the binding of selective cannabinoid receptor agonists from rat basophilic leukaemia RBL-2H3 and cerebellar granule cell membranes [20,21], but was inactive in binding experiments carried out with cells transfected with either CB1 or CB2 receptors [22,23].

### 3.3. Non-cannabinoid receptor-mediated actions

Like THC, anandamide exhibits some pharmacological actions independently of CB1, CB2 or CBn cannabinoid receptors. The 'endocannabinoid' inhibits non-competitively L-type $Ca^{2+}$ channels by interacting with 1,5-dihydropyridine and 1,5-benzothiaze-

pine and phenylalkylamine binding sites in rat cardiac and brain membranes and rabbit skeletal muscle membranes [60,61,193]. Anandamide and THC also inhibit Shaker-related voltage-gated $K^+$ channels [62]. These two actions were exerted in the low–medium μM range of concentrations. At much lower concentrations ($IC_{50}$ = 190 nM), anandamide, together with two synthetic cannabinoids, was shown to block 5-$HT_3$ receptor-mediated currents in rat nodose ganglion independently of G-protein activation [63]. 5–100 μM concentrations of anandamide were found to potentiate $Ca^{2+}$/phosphatidylserine activation of rat brain protein kinase C isozymes [64]. Interestingly, when the assay was carried out in the presence of dioleoylglycerol, which further activates the enzyme, anandamide inhibited protein kinase C-catalysed protein phosphorylation, suggesting a possible interaction as a partial agonist with the diacylglycerol binding site of the enzyme. Finally, anandamide was very recently found to positively modulate NMDA-mediated $Ca^{2+}$ currents in cells that do not express cannabinoid receptors, or where these receptors had been functionally inactivated by pertussis toxin pretreatment. A direct interaction with the NMDA glutamate receptor was suggested [65].

## 4. Formation and inactivation of 'endocannabinoids' and other fatty acid derivatives with cannabimimetic properties

### 4.1. Analytical studies and tissue distribution

Following the finding of anandamide, novel analytical methods for the purification and quantitative measurement of NAEs were developed based mainly on HPLC, gas chromatography (GC) and GC/mass spectrometry (MS) of various derivatives [66–72]. Preparation of the 4-(*N*-chloroformyl-methyl-*N*-methyl)amino-7-*N*,*N*-dimethyl-amino-sulphonyl-2,1,-3-benzoxa-diazole derivatives followed by HPLC and fluorometric detection provided the first highly sensitive quantitative method [66]. Another HPLC method exploited the preparation of the 1-anthroyl derivatives of anandamide [67]. Acetylation of the 2′-hydroxyl group or its derivatisation with either trimethyl-silyl-, *t*-butyl-dimethyl-silyl-, bis-pentafluorobenzoyl- or bis-pentafluoro-propionyl-groups were used for GC and GC/MS methods [68–70]. Anandamide was also directly quantitated without derivatisation by using liquid chromatographic (LC)-atmospheric pressure chemical ionisation MS or LC/MS/MS [71,72]. By using these methods, anandamide, in amounts ranging from about 5 to 150 pmol/g tissue, was identified and quantitated in human, bovine, ovine and rat brain, rat testis, skin, kidney and spleen, human spleen and heart, mouse uterus, murine leukocytes, human breast cancer and rat phaeochromocytoma (PC-12) cells [66–77]. In particular, in human and rat brain, the highest levels of anandamide were found in the hippocampus (148 and 29 pmol/g tissue, respectively) and striatum [71]. *N*-Palmitoylethanolamine, when measured, was almost always found in higher amounts than anandamide, together with other congeners. 2-Arachidonoylglycerol was quantitated in rat brain [29,78] and canine gut, pancreas and spleen [28,79], and was also found to be accompanied by several monoacylglycerols. Finally, large amounts (55–680 pmol/$10^7$ cells, corresponding to about 0.5–6 nmol/g tissue) of oleamide have been found in N18TG2 neuroblastoma [80], human breast cancer and rat phaeochromocytoma cells [77].

### 4.2. Biosynthesis of anandamides and other NAEs

The two routes suggested in the 1960s and 1980s [19] for the biosynthesis of saturated, mono- and diunsaturated NAEs, i.e. the direct and energy-free enzymatic condensation between the fatty acid and ethanolamine, and the phospholipase D-catalysed hydrolysis of *N*-acyl-phosphatidylethanolamines, have been shown to apply also to anandamide (Fig. 2). The first pathway occurs in mammalian brain particulate fractions independently of ATP and coenzyme A and in the presence of high μM concentrations of arachidonic acid and high mM concentrations of ethanolamine [81,82]. The enzyme responsible for this reaction was named anandamide synthase, was active at alkaline pH (8.5–10) and showed some selectivity for arachidonic acid and ethanolamine versus other fatty acids or amines. The synthase was found in both rabbit and rat brain, but also in rabbit liver, kidneys and lungs [81]. In rat brain, the highest synthase activity was in the hippocampus, followed by a moderate activity in the tha-

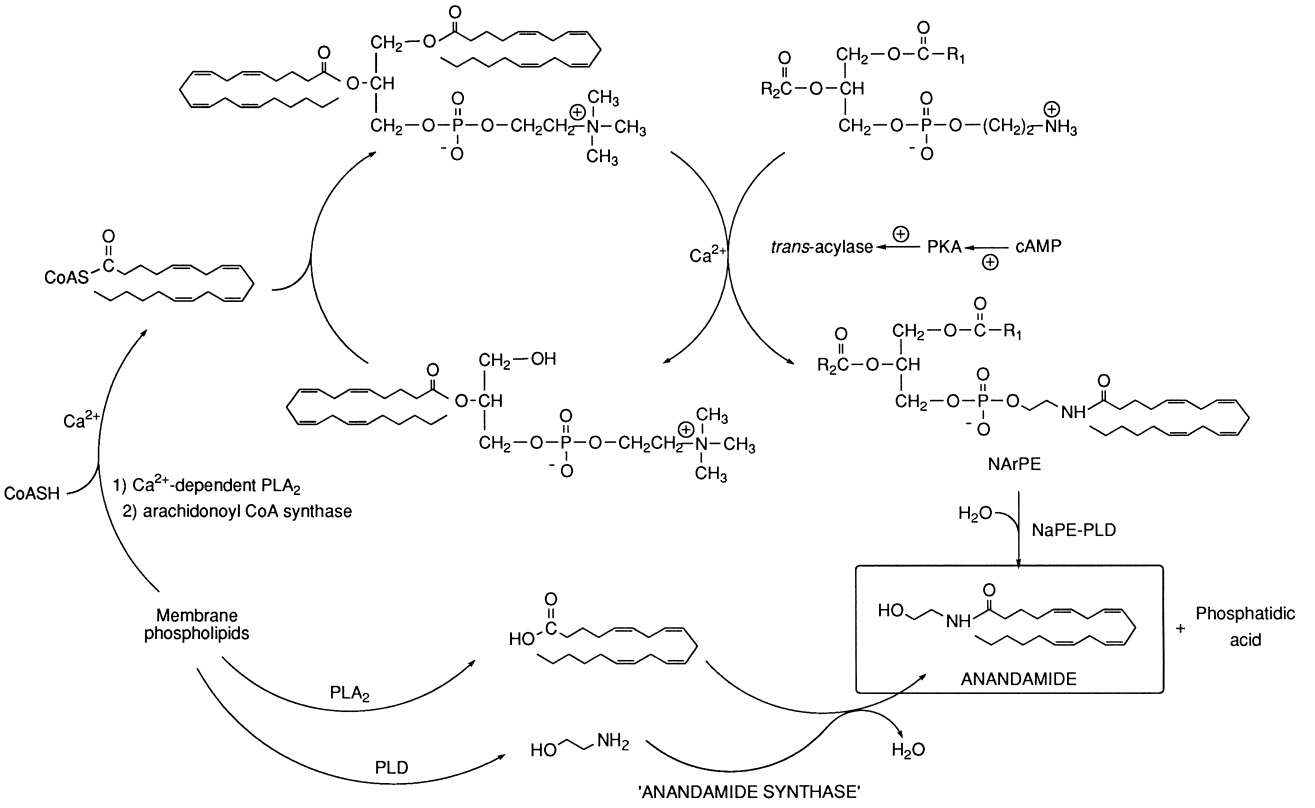


Fig. 2. Biosynthetic pathways for anandamide formation in cells and their possible regulation. PKA, cAMP-dependent protein kinase; NArPE, *N*-arachidonoyl-phosphatidylethanolamine; NAPE-PLD, *N*-acylphosphatidyl-ethanolamine-selective phospholipase D; $PLA_2$, phospholipase $A_2$.

lamus, striatum and frontal cortex [82]. As the cellular concentrations of arachidonic acid and ethanolamine are normally much lower than those necessary to the enzyme, it was suggested that 'anandamide synthase'-mediated anandamide biosynthesis would require the activation of the two phospholipases mostly responsible for arachidonic acid and ethanolamine release from membrane phospholipids, i.e. phospholipases $A_2$ and D ($PLA_2$ and PLD). Later, 'anandamide synthase' was partially purified from porcine brain [83] and shown to exhibit the same pH/temperature dependence and inhibition profiles as the enzyme catalysing anandamide hydrolysis to arachidonic acid and ethanolamine, named anandamide amidohydrolase (see below), with which the 'synthase' co-eluted after two chromatographic steps and shared the same tissue distribution. This finding, together with the high substrate concentrations required to observe an 'anandamide synthase' activity, suggested that this enzyme was an 'anandamide amidohydrolase' working 'in reverse' [83]. When the amidohydrolase was cloned and expressed in COS-7 cells, it was indeed found to catalyse the reverse reaction on incubation with high mM, and probably physiologically non-relevant, concentrations of ethanolamine [84,85]. To date no other 'anandamide synthase' has been characterised yet. This allows us to propose that the second pathway suggested for anandamide biosynthesis is the physiological route for the formation of the 'endocannabinoid', at least in the CNS. This pathway was shown to lead to the $PLA_2$-independent production of anandamide and other NAEs in intact rat neurones stimulated with membrane-depolarising agents (e.g. ionomycin, kainate and high potassium), and occurs through the phosphodiesterase-catalysed hydrolysis of a membrane phospholipid precursor, *N*-arachidonoyl-phosphatidylethanolamine (NArPE) [86]. Both NArPE and other *N*-acyl-phosphatidylethanolamines (NaPEs), with a *N*-fatty acid composition very similar to that of the NAEs co-synthesised with anandamide, but different from the free fatty acid composition, were characterised in rat neuronal and brain lipid fractions, and their turnover found to be in-

duced by ionomycin [86,87]. Moreover, a PLD-like enzyme catalysing the conversion of NArPE to anandamide was found in rat neuronal and brain homogenates [86,87]. This enzyme also recognised other NaPEs with an apparent affinity higher than NArPE [87], and is probably the same NaPE-selective PLD partially characterised from dog brain in 1983 [88]. A $Ca^{2+}$-dependent *trans*-acylase activity responsible for NArPE formation through the transfer of arachidonic acid from the *sn*-1 position of 1,2-*sn*-di-arachidonoyl-phosphatidylcholine (1,2-*sn*-di-arachidonoyl-PC) to phosphatidylethanolamine (PE) was also partially characterised [87]. The enzyme displayed optimal activity at pH = 7.0 and $K_m$ values of about 20 and 120 μM, respectively, for 1,2-*sn*-di-arachidonoyl-PC and PE, and could also catalyse the transfer to the latter compound of other fatty acids, thus possibly accounting for the formation of other NaPEs. In separate studies, the *trans*-acylase activity was also found in rat testes and N18TG2 cells [67,89], and its distribution in rat brain regions shown to moderately resemble that of anandamide, with levels highest in brainstem, and intermediate in the hippocampus, cortex, striatum, medulla and cerebellum [90]. The enzyme was suggested to be up-regulated, in rat central neurones, by cAMP-induced activation of protein kinase A (PKA), provided that intracellular [$Ca^{2+}$] is also raised [91]. Conversely, in PC-12 cells transformed with nerve growth factor into cells with a phenotype typical of sympathetic neurones, NAE and NaPE contents were not significantly different from those of untransformed PC-12 cells [77]. In any event, *trans*-acylase inhibition prevented $Ca^{2+}$-dependent NArPE formation in intact neurones [90]. This confirms that the NaPE-PLD-mediated pathway (which was suggested to take place also in N18TG2 and RBL-2H3 cells and J774 mouse macrophages, stimulated with antigen and/or ionomycin [75,89,92], as well as in sea urchin ovaries [93]) may be the physiological route for anandamide formation at least in neurones and some peripheral cells. The major drawback of this pathway, however, is represented by the low levels of 1,2-*sn*-di-arachidonoyl-PC found so far only in brain [87,90] and testes [94] (respectively 0.3% and 3.4% of total phosphatidylcholine species). Future studies will have to aim at understanding the regulation of the biosynthesis of this phospholipid which is likely to represent the rate-limiting step for anandamide formation in neurones. Interestingly, a recent study carried out in mouse peritoneal macrophages showed that the de novo formation of 1,2-*sn*-di-arachidonoyl-PC, which accounts for 1% of total PC, can be enhanced by $Ca^{2+}$ ionophore stimulation to up to 4% of total newly incorporated glycerol, possibly using arachidonic acid released by a $Ca^{2+}$-dependent $PLA_2$ [95] (Fig. 2). Whether a similar pathway also occurs in neurones remains to be established. On the other hand, the recent identification in the mouse uterus [96] of two *distinct* enzymatic activities for the synthesis and degradation of anandamide, together with the finding in the same tissue of anandamide in levels higher than NArPE, and up to 20 nmol/g tissue [74], may suggest that, at least in some peripheral tissues, the 'endocannabinoid' may be synthesised through the 'synthase' or other pathways.

### 4.3. Biosynthesis of 2-arachidonoylglycerol

Also the biosynthesis of 2-arachidonoylglycerol can be stimulated by ionomycin in neuronal cells [97]. Evidence for the $Ca^{2+}$-dependent formation and release of this 'endocannabinoid' was obtained for the first time in mouse neuroblastoma N18TG2 cells, where 2-arachidonoylglycerol biosynthesis (1) was accompanied by the formation of monooleoylglycerol, (2) occurred concomitantly with 1-acyl-2-arachidonoylglycerol (AArG) formation, (3) was potentiated by incubation of cells with exogenous $PLA_2$ (which, on the contrary, should have reduced the amount of phospholipid precursors for AArG and 2-arachidonoylglycerol), and (4) was not sensitive to the phospholipase C (PLC) general inhibitor neomycin sulphate [97,98]. However, apart from an abundant *sn*-1-diacylglycerol lipase catalysing AArG hydrolysis to 2-arachidonoylglycerol [89,97], neuroblastoma cell homogenates were found to contain also a $Ca^{2+}$-dependent PLC capable of slowly but significantly causing the sequential formation of AArG and 2-arachidonoylglycerol from 1,2-*sn*-di-arachidonoyl-PC [89]. A subsequent study confirmed that 2-arachidonoylglycerol can be released from rat cortical neurones, but not astrocytes, in a $Ca^{2+}$-dependent fashion and through the intermediacy of AArGs. Differently from N18TG2 cells, however, in this study a phosphoinositide (PI)-selective PLC

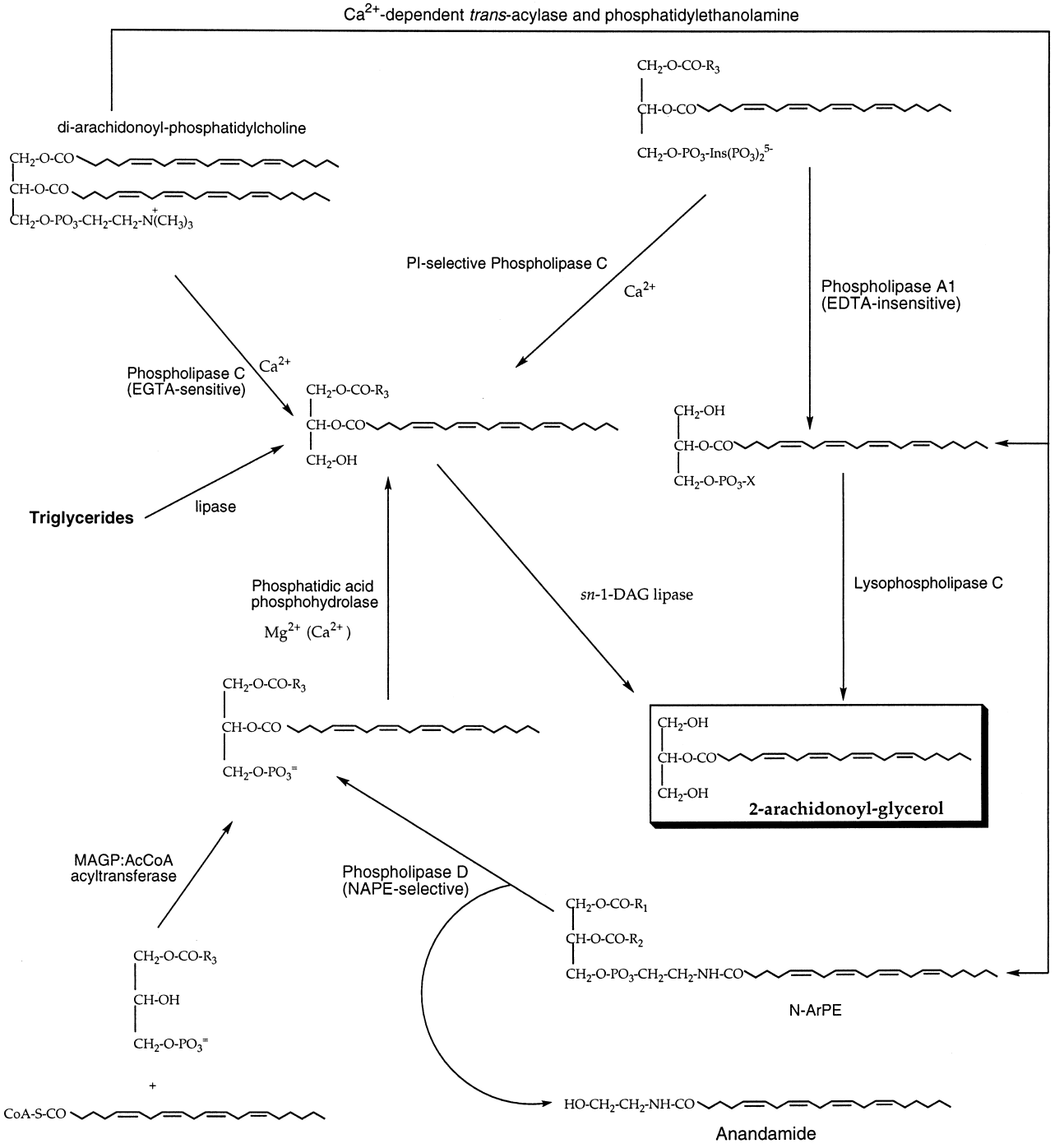


Fig. 3. Possible pathways for 2-arachidonoylglycerol biosynthesis in cells. Two types of *sn*-2-arachidonate-containing glyceride precursors have been suggested for 2-arachidonoylglycerol: diacylglycerols and lysophosphoglycerides. The latter can be produced either from the action of phospholipase A1, or during *trans*-acylase-catalysed *N*-acylphosphatidyl-ethanolamine (NAPE) biosynthesis. PI, phosphoinositide; DAG, diacylglycerol; NArPE, *N*-arachidonoyl-phosphatidylethanolamine; MAGP, monoacylglycerol-3-phosphate; AcCoA, acylcoenzyme A.

was suggested to be responsible for the AArG-mediated formation of 2-arachidonoylglycerol [78]. From these data it can be suggested that the diacylglycerol species serving as precursors for the biosynthesis of the 'endocannabinoid' may be formed either through typical PLC-mediated pathways or via de novo for-

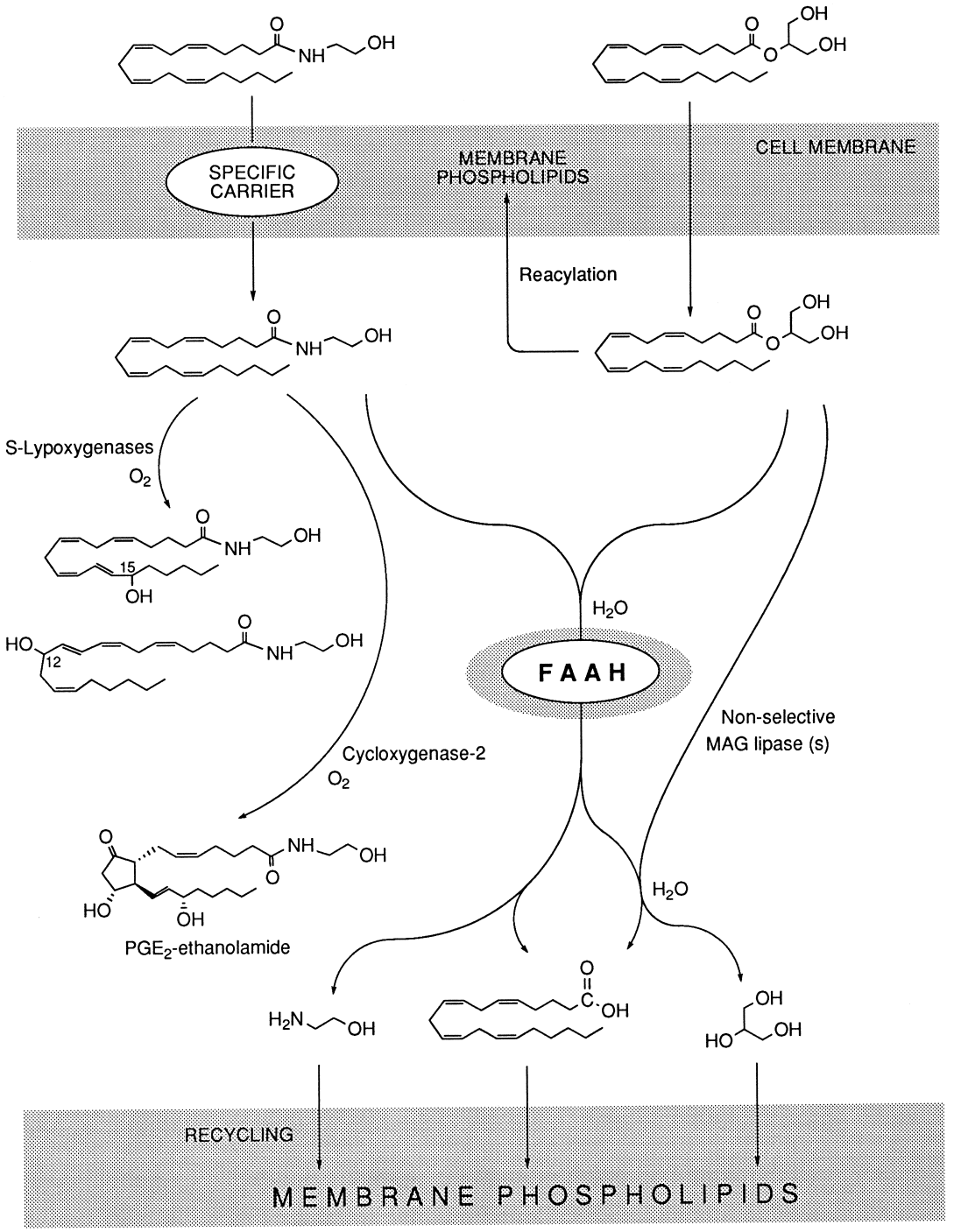


Fig. 4. Possible mechanisms for the inactivation of 'endocannabinoids' by cells. A specific carrier protein has been identified for anandamide uptake by cells, but no evidence for such a mechanism exists yet for 2-arachidonoylglycerol, which, instead, can be acylated into membrane phospholipids. Anandamide can also be oxidised using enzymes of the 'arachidonate cascade', whereas both FAAH and non-selective lipases may contribute to 2-arachidonoylglycerol hydrolysis. $PGE_2$, prostaglandin $E_2$.

mation, depending on whether $Ca^{2+}$ influx, and the subsequent neurone depolarisation, leads to PLC activation or not (Fig. 3). Two further $Ca^{2+}$-dependent pathways, occurring concomitantly with anandamide formation, can also be hypothesised. Firstly, 1-lyso-2-arachidonoyl-PC, a by-product of NArPE biosynthesis (Fig. 2), can be directly converted into 2-arachidonoylglycerol by an EDTA-insensitive lysoPLC

identified in N18TG2 cell homogenates [89]. Secondly, *sn*-2-arachidonate-containing phosphatidic acid, produced as a by-product of anandamide formation, may generate AArGs required for 2-arachidonoylglycerol formation (Fig. 3). This second alternative route still needs to be investigated. However, it is worthwhile noting that electrical stimulation of hippocampal slices led to selective and $Ca^{2+}$-dependent 2-arachidonoylglycerol formation without increasing the basal levels of anandamide [78]. Therefore it is possible that the two 'endocannabinoids' are synthesised independently of each other, at least in some brain regions. Nevertheless, biosynthetic routes for 2-arachidonoylglycerol in addition to that utilising PI-selective PLC, such as the de novo or PLC-catalysed formation of AArG pools which are not used in protein kinase C (PKC)-mediated signal transduction, or the one-step conversion of 1-lyso-2-arachidonoyl-phospholipids into the 'endocannabinoid', should be investigated in the future. In fact, if 2-arachidonoylglycerol is formed uniquely from the hydrolysis of AArGs produced within a framework of receptor-evoked *trans*-membrane signalling events, the initiation of an extracellular cannabimimetic signal would inevitably follow such events. The subsequent input delivered to cells might represent a negative feedback signal, as suggested by Sugiura et al. [44], or a confusing 'go'-'stop' message. A typical example would be the modulation of hippocampal long-term potentiation, which is induced by AArGs through PKC activation [99] and inhibited by 2-arachidonoylglycerol [78]. It is possible that, apart from alternative biochemical routes and different diacylglycerol pools, also different PLC isozymes are used for AArG-mediated signalling and 2-arachidonoylglycerol biosynthesis. Following this line of thought, it is worthwhile noting that anandamide is formed by a PLD enzyme apparently distinct from the PE- and PC-selective PLD involved in signal transduction [88], and that several biosynthetic routes (recently reviewed in [100]) have been proposed for arachidonic acid, a molecule active of its own and the precursor of several bioactive metabolites.

### 4.4. Oleamide and other fatty acid primary amides

Two biosynthetic routes have been proposed for oleamide and its congeners, such as arachidonamide and erucamide, which represent a class of bioactive fatty acid amides whose mechanism of action has not been clarified yet but may have to do with prolongation and potentiation of 'endocannabinoid' activity (see below). The energy-free condensation of ammonia and fatty acids to primary amides catalysed by rat brain microsomes was shown to lead to oleamide biosynthesis [101], but this reaction is very likely mediated by anandamide amidohydrolase working 'in reverse' [84] (see below), and was recently shown not to contribute to the formation of oleamide in intact N18TG2 cells [80]. Another hypothesis is that oleamide is formed through the action of peptidylglycine α-amidating monooxygenase (PAM), the enzyme responsible for the C-terminal amidation of peptides and proteins, which was recently shown to convert *N*-myristoyl- and *N*-oleoyl-glycine respectively into myristoylamide and oleamide [102,103]. According to this hypothesis, *N*-acylglycines are synthesised in the liver or kidneys from acylCoA and glycine through the action of acylCoA:glycine *N*-acyltransferase, an enzyme which is absent in the brain. *N*-Oleoylglycine would then cross the blood-brain barrier and be converted into oleamide by PAM, which is present in the cerebrospinal fluid and hypothalamus [102]. Another possibility is that *N*-oleoylglycine is converted into oleamide in blood, and then transported to tissues. However, recent studies have shown that oleamide is a major lipid component of neuronal and peripheral cells in culture [77,80], which suggests that oleamide formation may occur independently of *N*-oleoylglycine, unless one assumes that this compound is present in the media used to culture the cells. Moreover, a compound with the same chromatographic behaviour as oleamide was found to be produced from N18TG2 cells incubated with [$^{14}$C]oleic acid, in a fashion that seemed to depend on how much oleic acid had been incorporated into membrane phospholipids [80]. Thus, further studies are needed in order to clarify the issue of fatty acid primary amide biosynthesis.

### 4.5. Inactivation of 'endocannabinoids'

The first step in both anandamide and 2-arachidonoylglycerol inactivation is their rapid diffusion into cells [86,104–106] (Fig. 4). Although the lipophilicity of the two compounds allows them to passively dif-

fuse through the cell membrane, facilitated transport mechanisms have been described for anandamide in rat cortical neurones [86,105], RBL-2H3 basophils [75], cerebellar granules [104] and astrocytes [105], with $t_{1/2}$ values ranging from 2.5 and 7 min. These mechanisms seem to be mediated by specific $Na^+$-independent carrier proteins in as much as they are (1) saturable, (2) temperature-dependent, (3) selective, and (4) inhibited by various more or less selective agents, such as phloretin, phenylmethylsulphonyl fluoride (PMSF) and *N*-ethylmaleimide (NEM), or anandamide analogues, like the naturally occurring *N*-oleoylethanolamine, or the synthetic compounds *N*-arachidonoylbenzylamine, *N*-arachidonoylpropylamine and *N*-(4-hydroxyphenyl)arachidonoylamine (AM404). The latter compound selectively inhibits anandamide uptake into rat cortical astrocytes and neurones, and was used to demonstrate a functional role of this facilitated transport mechanism in the termination of anandamide action in vivo and in vitro [105]. No evidence exists for a carrier-mediated uptake of 2-arachidonoylglycerol, which, however, rapidly diffuses into cells ($t_{1/2}$ values ranging from 6 to 27 min) and is partly esterified into membrane phospholipids [106]. Once inside the cell, both anandamide and 2-arachidonoylglycerol are degraded mostly through the enzymatic hydrolysis of their amide or ester bonds, thereby yielding arachidonic acid, which is immediately re-incorporated into membrane phospholipids, and ethanolamine or glycerol, respectively [86,75,106] (Fig. 4).

### 4.6. *Anandamide amidohydrolase/fatty acid amide hydrolase: an enzyme 'for all seasons'*

The enzyme responsible for anandamide hydrolysis, originally named 'anandamide amidohydrolase', or 'amidase', has been widely studied. It has been characterised from rat, mouse and porcine brain particulate fractions, and shown to be optimally active at alkaline pH (8.5–10), quite selective for anandamide versus other *N*- acylethanolamines, and inhibited by typical serine and cysteine protease inhibitors such as PMSF, *p*-hydroxy-mercuribenzoate, *p*-bromo-phenacyl-bromide and NEM [83,107–110], as well as by the $PLA_2$ inhibitor arachidonoyl-trifluoromethylketone (ATFMK) [111]. The enzyme displayed a tissue distribution (with the highest activity in liver, brain and lungs), and a pH dependence profile almost identical to those of an amidohydrolase previously reported to catalyse the hydrolysis of oleoylethanolamide in dog brain [108,109,112]. In rat brain the enzyme was mostly concentrated in the striatum, hippocampus, substantia nigra and thalamus [108]. Thus the tissue distribution of 'anandamide amidohydrolase' appeared to perfectly mirror that of 'anandamide synthase' [81,82]. Moreover, the two activities were co-purified from porcine brain, thus strongly suggesting that they were due to the same enzyme [83]. Final confirmation to this hypothesis came when the enzyme catalysing the hydrolysis of the sleep-inducing factor oleamide was purified to homogeneity, cloned and sequenced [113]. In fact, previous studies carried out in N18TG2 cells had shown that 'anandamide amidohydrolase' could also recognise oleamide [114]. Therefore, when the cDNA sequence of 'oleamide hydrolase' was determined, it was possible to overexpress an appropriately modified cDNA probe into COS-7 cells. This made it possible to demonstrate that the enzyme catalysed (a) the hydrolysis of anandamide [113] and (b) the hydrolysis 'reverse reaction', i.e. the synthesis of anandamide from ethanolamine and arachidonic acid, in the presence of high concentrations of these two substrates [84,85]. Using transfected COS-7 cells it was also possible to show that the amidohydrolase also recognises other fatty acid amides [113] and methyl esters [84], as well as catalysing the synthesis of oleamide and arachidonamide from high concentrations of ammonia and oleic or arachidonic acid [84] (Fig. 5). Since the likely physiological function of this enzyme, as suggested also by the presence of a significant homology with other amide hydrolase enzymes, was to catalyse the hydrolysis rather than the synthesis of several fatty acid amides, the new name 'fatty acid amide hydrolase' (FAAH) was proposed [113]. The sequence analysis of FAAH showed also a hydrophobic region predicted to assume a trans-membrane α-helical conformation, and a consensus class II SH3 domain binding sequence, which suggested that other proteins may interact with FAAH, thereby regulating its activity and/or subcellular localisation. Southern and Northern blots of DNA and mRNA from various rat tissues, probed with an internal 800-bp fragment of FAAH cDNA, confirmed previous data on the tissue distribution of this protein, whose levels are highest in the liver, brain and kidneys,

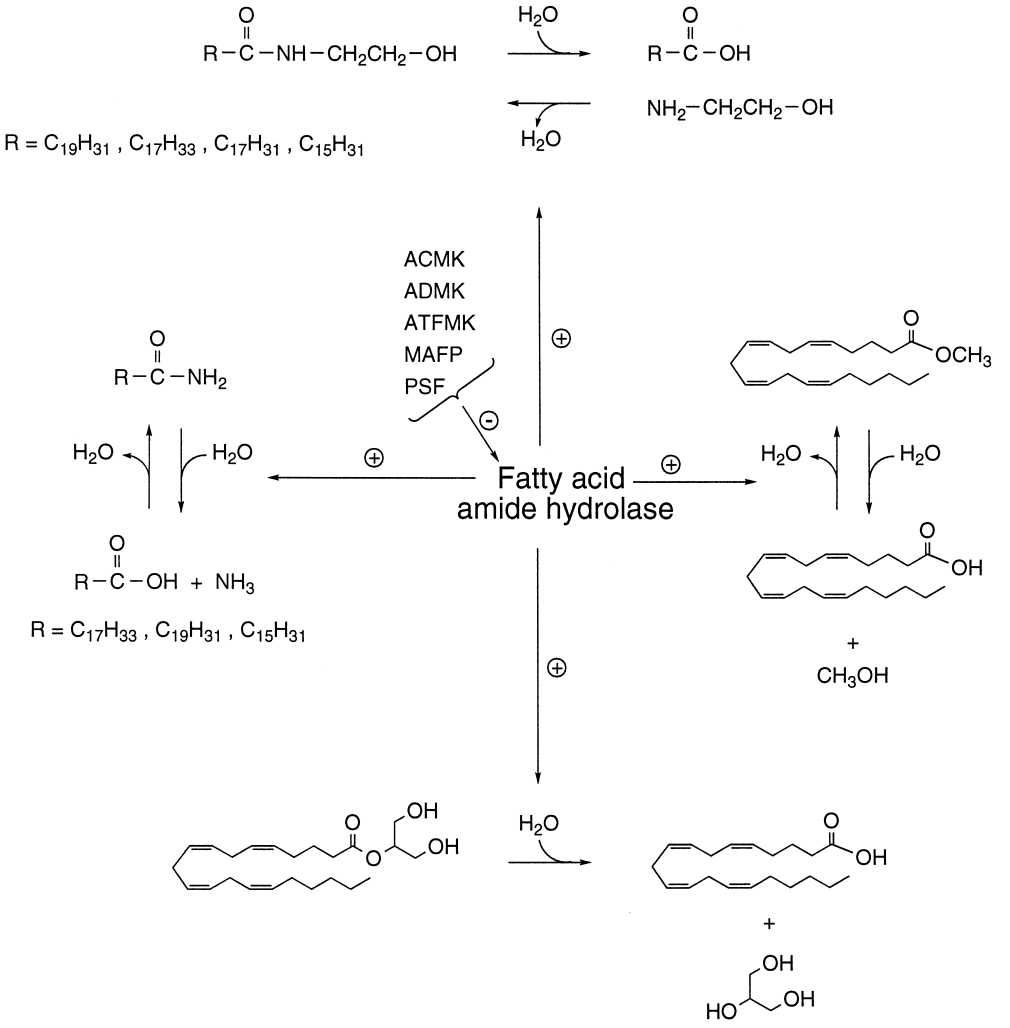


Fig. 5. Multiple functions for fatty acid amide hydrolase. The enzyme can catalyse both the hydrolysis of fatty acid primary amides, ethanolamides and methyl esters and, in the presence of high substrate concentrations, the reverse reaction. The FAAH-catalysed hydrolysis of 2-arachidonoylglycerol, but not the condensation between glycerol and arachidonic acid, can also be catalysed by recombinant FAAH. ACMK, arachidonoylchloromethylketone; ADMK, arachidonoyldiazomethylketone; ATFMK, arachidonoyltrifluoromethylketone; MAFP, methylarachidonoylfluorophosphonate; PSF, palmitylsulphonylfluoride.

measurable in testes and lung, low in the spleen and undetectable in the skeletal muscle and heart [113]. In rat brain, FAAH mRNA, like 'anandamide amidohydrolase' activity and CB1 receptors, is expressed in the neocortex, amygdala, hippocampus, thalamus and in confined regions of the hypothalamus, brainstem and cerebellum [191]. Mouse and human FAAH were also recently cloned [115], and their structures found to exhibit extensive homology with that of the rat enzyme, even though some significant differences between the substrate specificity and tissue distribution of rat and human FAAH were observed. The possible identity of 'anandamide amidohydrolase' with FAAH was confirmed also by more recent studies [77,116] where the amounts of FAAH transcripts and the $V_{max}$ values for 'anandamide amidohydrolase' in several rat tissues and tumour cell lines were compared and found to correlate. FAAH-like enzymes have been described also in other peripheral tissues such as sea urchin ovaries [93], mouse uterus [96], ocular tissues [117] and RBL-2H3 basophils [75]. In the latter cell type, FAAH, unlike the enzyme from brain and neuronal cells, displayed a high affinity toward *N*-palmitoylethanolamine. The possibility of this being due to the existence of a FAAH isoform needs further investigation.

The potential physiological importance of FAAH is increased further by very recent findings obtained independently by two groups of researchers. Stemming from the observation that FAAH from rat intestine was inhibited by 2-arachidonoylglycerol [116], and that the recombinant enzyme expressed in COS-7 cells could also catalyse the hydrolysis of arachidonoyl methyl ester, Yamamoto and colleagues tested microsomal preparations from COS-7 cells transfected with FAAH cDNA and found that they could efficiently convert 2-arachidonoylglycerol into arachidonic acid and glycerol [118]. The reaction was not observed in untransfected control cells, and was inhibited by an excess of anandamide as well as by methylarachidonoylfluorophosphonate (MAFP), a potent inhibitor of FAAH [119,120]. Partially purified 'anandamide amidohydrolase' from porcine brain also hydrolysed 2-arachidonoylglycerol. Independent work carried out in our laboratory with the aim of characterising the lipase responsible for 2-arachidonoylglycerol hydrolysis in N18TG2 and RBL-2H3 cells showed that this enzyme exhibited the same pH dependence profile as FAAH and, when assayed at pH 9.5, it co-eluted with FAAH activity after two chromatographic steps [106]. This lipase was inhibited by typical FAAH inhibitors as well as by anandamide, and showed no selectivity towards the 2- or 1(3)-isomers of the monoglyceride, although, like FAAH, it did discriminate between polyunsaturated and saturated substrates by exhibiting much higher affinity for the former compounds [98,106]. Moreover, a FAAH inhibitor (ATFMK) enhanced the levels of 2-arachidonoylglycerol formed de novo in intact N18TG2 cells stimulated with ionomycin. These data, taken together, strongly suggest that FAAH may be one of the enzymes responsible for the physiological degradation of 2-arachidonoylglycerol (Fig. 5).

### 4.7. Specific fatty acid amide hydrolase inhibitors

Recently, FAAH inhibitors with different mechanisms of action have been obtained from the derivatisation of arachidonic acid and/or oleic acid with functional groups potentially reactive towards catalytically active serine and cysteine residues. Such compounds may be particularly useful for the development of new drugs in view of the multi-functional role proposed for FAAH. Diazomethyl and chloromethyl derivatives of arachidonic acid [119] were found to potently inhibit FAAH from different sources with $IC_{50}$ values ranging between 2 and 23 μM. These compounds probably form reversible tetrahedral adducts with the active site –SH or –OH groups, as previously suggested for the acyltrifluoromethylketones [111]. Several oleic acid derivatives were also tested, and the most potent compound was trifluoromethylketone [121], which was used for the purification of FAAH [113]. MAFP is the most potent FAAH inhibitor so far described, with $IC_{50}$ values between 1 and 4 nM, and forms a covalent, enzymatically inactive adduct with the enzyme [118–120]. Acylsulphonylfluorides are also very potent irreversible inhibitors of anandamide enzymatic hydrolysis with $IC_{50}$ values in the low nM range [122]. However, most FAAH inhibitors developed so far are not totally selective towards this enzyme since they either also inhibit other enzymes involved in the arachidonate cascade or bind to CB1 receptors. Thus, ATFMK and (*E*)-6-(bromomethylene)-tetrahydro-3-(1-naphthalenyl)-2-*H*-pyran-2-one inhibit cytosolic $PLA_2$ at the same concentrations used to inhibit FAAH [123,124], ibuprofen does not discriminate between cyclooxygenase-2 and FAAH [125], and arachidonoyl-diazomethylketone also inhibits human leukocyte 5-lipoxygenase [126]. MAFP, on the other hand, is active on cytosolic $PLA_2$ only at concentrations (μM) much higher than those (nM) required to inhibit FAAH, but it covalently binds to the CB1 receptors when using doses in the high nM range [120,127]. The acylsulphonylfluorides with 14, 16 and 18 carbon atoms also inactivate CB1 receptors, but only at concentrations 500–1000 higher than those used for FAAH inhibition [122]. These compounds need to be tested on other enzymes before their employment as selective FAAH inhibitors can be suggested.

### 4.8. Other pathways for 'endocannabinoid' catabolism

Apart from amide bond hydrolysis, anandamide has also been shown to undergo, in tissue homogenates or purified enzyme preparations, another series of metabolic reactions consisting of the oxidation of its double bonds with subsequent formation of hydroperoxy- and hydroxy-anandamide derivatives (Fig. 4). These reactions are catalysed by enzymes of the arachidonate cascade such as cytochrome

P450 hydroxylases [128] or by lipoxygenase enzymes [129–131]. More recently, anandamide was shown to be recognised also by cyclooxygenase-2, but not cyclooxygenase-1, thereby being converted into a non-cannabimimetic prostaglandin $E_2$-ethanolamide [132]. Even more unusual enzymes involved in arachidonic acid and polyunsaturated fatty acid metabolism may recognise anandamide, as shown, for example, for an algal 'arachidonic acid conjugated-triene isomerase' which forms the conjugated 5*Z*,7*E*,9*E*-triene derivative, a compound which was found to retain significant CB1 receptor binding activity [133]. No analogous reaction for 2-arachidonoylglycerol, although feasible in principle, has been observed yet, whereas oleamide and anandamide C18:2 analogues were recently found to serve as substrates for soybean lipoxygenase [134]. So far the only preliminary indication that such reactions occur in intact cells under pseudo-physiological conditions is the formation of 12(*S*)- and 15(*S*)-hydroxy-anandamide by human neutrophils [131]. Therefore, the possible physiological significance of such reactions still needs to be supported by further experimental data. Since the FAAH-mediated pathway seems to be the predominant mechanism for anandamide and 2-arachidonoylglycerol degradation, it is possible that enzymatic oxidation of 'endocannabinoids' intervenes in those tissues where FAAH is not very abundant. Moreover, since some hydroxy derivatives of anandamide retain significant cannabinoid binding activity [129–132], or even compete with anandamide for FAAH [134], it is also possible that such pathways are not used by cells to inactivate anandamide, but rather by anandamide to competitively inhibit the formation of bioactive arachidonic acid oxidation products. This hypothesis may explain some anandamide non-cannabinoid receptor-mediated actions, but requires experimental verification.

### *4.9. Endogenous inhibitors of 'endocannabinoid' inactivation*

Recent findings suggest that natural analogues of anandamide and 2-arachidonoylglycerol, which coexist or are even co-synthesised with the two 'endocannabinoids' in equal or higher amounts, may play a physiological role as endogenous 'enhancers' and 'prolongers' of 'endocannabinoid' action by inhibiting 'endocannabinoid' inactivation. The capability of *N*-oleoylethanolamine, one of the most abundant NAEs in neurones, to inhibit anandamide uptake has been mentioned already [104]. This metabolite and *N*-linoleoylethanolamine are also efficient inhibitors of anandamide enzymatic hydrolysis [114,135], while the 13(*S*)-hydroxy derivative of the latter compound, whose presence in tissues has not been described yet, is an even more potent competitive inhibitor of FAAH [134]. More interestingly, oleamide, which is known to compete with anandamide for FAAH active site [113,114], was recently suggested to owe some of its pharmacological actions, including analgesia, inhibition of locomotor activity and hypothermia, to an increase of endogenous anandamide levels [26]. Accordingly, oleamide enhanced the binding of anandamide to CB1 receptors, and, at low doses, significantly potentiated anandamide anti-proliferative action on human breast cancer cells [77], as well as anandamide effects in the 'tetrad' of mouse behavioural tests [26]. Also the weak cannabimimetic activity observed for *N*-oleoylethanolamine and *N*-linoleoylethanolamine in those tests [136] may be due to potentiation of a tonal action by endogenous anandamide in the brain. Finally, monoacylglycerols such as 2-palmitoyl- and 2-linoleoyl-glycerol, which show no cannabimimetic activity even at very high doses, were found to enhance 2-arachidonoylglycerol's functional activation of CB1 and CB2 receptors, and to potentiate 2-arachidonoylglycerol action in the 'tetrad' of tests. However, of the two compounds, only the unsaturated monoglyceride was active as an inhibitor of 2-arachidonoylglycerol uptake and enzymatic hydrolysis [79]. Therefore, simple inhibition of 'endocannabinoid' degradation may not be the only factor underlying the activity-enhancing action of 'entourage' 'endocannabinoid' congeners. Further studies will be required in this direction, also in view of the recent finding that anandamide and 2-arachidonoylglycerol may share the same inactivating enzyme, and appear to inhibit each other's degradation [106,118].

## 5. Possible physiopathological importance of the 'endocannabinoids'

Despite the several studies carried out on the phar-

macological activity of both plant and endogenous cannabinoids, the physiological role of the 'endogenous cannabinoid system' is still a subject for speculations. Probably due to their rapid degradation in vivo [137], it has not always been possible to extend to 'endocannabinoids' the several pharmacological actions previously established for THC and other synthetic cannabinoids. The use of more stable anandamide analogues, such as (*R*)methanandamide [138], has recently made it possible to observe an activity more consistent with that of classical cannabinoids [192], while the development and testing, in an increasingly larger number of studies, of the selective CB1 antagonist SR 141716A has recently made it possible to gain some knowledge on the possible participation of 'endocannabinoids' in the 'tonal' control of certain functions. However, very little is yet known on the correlation of certain physiopathological responses with the production and inactivation of 'endocannabinoids' and the expression/activity of the enzymes involved. From the limited data available, the general impression is that the ECS may be a tuning system of numerous finely regulated physiological functions even in lower organisms, and that its importance is not limited to the CNS but extends to several peripheral processes, also outside the nervous system. It is possible that disruption of this regulatory system may cause and/or contribute to some disorders in both central and peripheral tissues [139].

### 5.1. Central nervous system

The several intracellular actions of 'endocannabinoids' in neurones, outlined above, may allow these compounds to exert a neuromodulatory role on neurotransmitter release/activity. Pre-synaptic inhibition of $Ca^{2+}$ channels may impair neurotransmitter release, whereas activation of $K^+$ channels may reduce the likelihood of action potential initiation/propagation. CB1-mediated modulation of adenylate cyclase may influence slow neurotransmission, neurotransmitter synthesis and neuronal plasticity. Since the highest concentrations of CB1 receptors and/or anandamide and/or FAAH have been found in the thalamus, hippocampus, cortex, striatum, substantia nigra and cerebellum, a role of the ECS in the control of cognitive and motor responses has been proposed. Accordingly, anandamide was found to inhibit electrically evoked dopamine release from rat striatal slices [140], to inhibit nigrostriatal dopamine synthesis [141] and to induce motor inhibition in rats through interference with dopaminergic and GABAergic transmission [141,142]. Moreover, anandamide potentiates $GABA_A$-mediated catalepsy in the globus pallidus [143], whereas SR 141716A did not decrease catalepsy induced by $D_1/D_2$ receptor blockade [144]. Anandamide impairs working memory [145] and memory consolidation in mice [146], whereas SR 141716A improves memory in rodents [147]. These effects may be due to interference with acetylcholine release which is inhibited by the synthetic cannabinoid WIN 55, 212-2 and enhanced by SR 141716A [148]. Moreover, 2-arachidonoylglycerol and/or anandamide inhibit hippocampal long-term potentiation [78,149] and long-term transformation [150]. Pre-synaptic, CB1-mediated blockade of glutamatergic synaptic transmission by anandamide in hippocampal neurones [151] may underlie both its inhibitory effect on long-term potentiation and its proposed neuroprotective function against neurotoxicity. The latter was suggested by the finding of increased levels of anandamide following cell death [69–71,152], as well as by its inhibition of NMDA receptor-mediated $Ca^{2+}$ influx in cortical and cerebellar slices [65]. Finally, CB1-mediated activation by anandamide of neuronal focal adhesion kinase in hippocampal neurones [46] may suggest for the 'endocannabinoid' a more general role in synaptic plasticity.

### 5.2. Hypothalamic functions

Even though no data are available on hypothalamic/pituitary 'endocannabinoids', the presence of a sparse but homogeneous population of CB1 receptors in the hypothalamus, and the capability of anandamide to induce c-*fos* activation in hypothalamic neurones may imply a role of the ECS in the tuning of hypothalamic functions, in particular the control of the secretion of pituitary hormones [153–157]. The activation of the release of corticotropin-releasing hormone, by causing the secretion of adrenocorticotropic hormone from the pituitary, may lead to the enhancement of corticosol production from the adrenal gland, as observed following intracerebroventric-

ular injection of anandamide [153]. Likewise, the release of other pituitary hormones, such as prolactin and the luteinising, follicle-stimulating and growth hormones, may be inhibited by anandamide acting on dopaminergic neurones at the level of the hypothalamus [155–157]. 'Endocannabinoids' may interact with hypothalamic thermoregulatory centres, thus explaining their hypothermic effects. Moreover, the wake/sleep cycle could also be regulated by the ECS, as suggested by a recent study showing that SR 141716A can prolong the time spent awake by rats [158], and by the fact that the sleep-inducing oleamide may act through anandamide [26].

### 5.3. Sensory nervous system

Recently a facilitatory action of THC on the release of dynorphins acting at either κ- or δ-opioid receptors has been described [159], but this mechanism seems to underlie the well-known antinociceptive actions of THC and not those of anandamide. Indeed, similarities and differences between the analgesic actions of exogenous and endogenous cannabinoids have been pointed out [160]. However, the finding that SR 141716A and antisense oligonucleotides against CB1 receptor mRNA can induce a significant thermal hyperalgesia may suggest that 'endocannabinoids' tonically regulate thermal nociceptive transmission [161]. Ongoing studies on the co-localisation of CB1 receptors with either substance P/glutamate-, calcitonin gene-related peptide- and endorphin-containing sensory fibres [162] may provide a clue as to the release/action of which sensory neurotransmitter is modulated by antinociceptive 'endocannabinoids'. Interestingly, a very recent study [201] showed that anandamide inhibits carrageenan-induced thermal hyperalgesia in rats and capsaicin-evoked release of calcitonin gene-related peptide from isolated rat hindpaw skin.

### 5.4. Autonomic nervous system and vasculature

Anandamide was shown to inhibit guinea pig small intestine and mouse urinary bladder and vas deferens contractions by a pre-synaptic, CB1-mediated inhibitory action on the release of acetylcholine from autonomic fibres [163–165]. Hypotension and bradycardia induced by anandamide were also shown to be due, at least in part, to a pre-synaptic action on CB1 receptors and to the subsequent inhibition of noradrenaline release from post-ganglionic sympathetic nerve terminals innervating the heart and vasculature [166–168]. Alternatively, inhibition of noradrenaline release from sympathetic neurones may be mediated by NO produced by nearby endothelial cells through a CB1-mediated action, as recently shown for the renal sympathetic nerves innervating the renal arteries [73]. Another possibility is that arachidonic acid and eicosanoids derived from anandamide hydrolysis are responsible for anandamide's hypotensive actions, as in bovine coronary artery where the involvement of prostacyclin and epoxyeicosatetraenoic acids has been suggested [196]. In this context, it may be worthwhile noting that there is now some pharmacological evidence that the endothelium-derived hyperpolarising factor (EDHF), whose structure has remained elusive since its discovery (for a recent review [169]), may be an 'endocannabinoid'. The induction of EDHF-mediated relaxations in the rat mesenteric arterial bed was blocked by SR 141716A, and was accompanied by the formation of an arachidonate metabolite co-eluting with anandamide on thin-layer chromatography. Moreover, synthetic anandamide was found to relax the mesentery through a hyperpolarising mechanism [170], and both anandamide- and EDHF-induced relaxations were inhibited by the putative EDHF (and cytochrome P450) inhibitors chlortrimazole and proadifen [171]. However, in a different study, carried out in the same tissue preparation, the relaxing actions of EDHF and anandamide were found to respond differently to the $K^+$ channel inhibitor apamin [172]. Two recent studies, carried out in rat isolated mesenteric and hepatic arteries, have pointed out the pharmacological similarities and differences in the hypotensive actions of EDHF and anandamide [194,195], and thus the issue of the chemical nature of the former compound has not been clarified yet. It may be worthwhile noting, however, that human vascular endothelial cells were recently found to generate and release 2-arachidonoylglycerol in response to thrombin or $Ca^{2+}$ ionophore stimulation [197]. Finally, a hypotensive action by anandamide preparations was also observed on ocular blood pressure [173,174]. In some studies an initial rise in ocular blood pressure was observed which

could be avoided by using stable anandamide analogues, such as *R*-α-isopropylanandamide, instead of anandamide [198].

### 5.5. Immune system and cytoprotection

'Endocannabinoids', by binding to both CB1 and CB2 receptors, and through the inhibition of cAMP formation or the activation of MAPK and cytosolic $PLA_2$, may act as chemical signals between different immune cells or between sensory fibres and blood cells. Basophils and mast cells, activated by IgE receptor cross-linking and/or $Ca^{2+}$ influx during inflammatory reactions, may release *N*-palmitoylethanolamine and anandamide (as shown for RBL-2H3 cells [75]), together with histamine, serotonin and leukotrienes. While the latter mediators produce vascular permeability and chemotaxis, *N*-palmitoylethanolamine behaves as an anti-inflammatory mediator in vivo [175] and in vitro [20] by acting as an autacoid signal on basophils and mast cells and inhibiting their degranulation. Anandamide, which can also be produced by macrophages [75,76,92,182], was found to counteract this effect [20], and at the same time to activate basophil/mast cell and macrophage arachidonate release [56,75,176], thus potentially leading to the formation of leukotrienes and prostaglandin $E_2$ which may have opposing actions on immune cell functionality. Moreover, by inhibiting lymphocyte proliferation [177] the 'endocannabinoid' might directly down-regulate the immune response. Given the low ability of anandamide to functionally activate CB2 receptors [178], 2-arachidonoylglycerol, which also inhibits lymphocyte proliferation [30], might act as a natural substitute for anandamide on target cells which express CB2 but not CB1 receptors. Also oleamide suppresses lymphocyte proliferation [27] and may act as an enhancer of anandamide (and 2-arachidonoylglycerol) immunosuppressive action. Anandamide also inhibits killing of tumour necrosis factor (TNF)-sensitive cells [179], as well as the *N*-formyl-Met-Leu-Phe-induced production of interleukins 4, 6 and 8, TNF-α and interferon-γ, by macrophages [176]. *N*-Palmitoylethanolamine exerts a similar inhibition of cytokine release, except for a non-significant effect on TNF-α and interferon-γ release [176]. On the other hand, a CB2-mediated facilitatory action on interleukin-3 and haematopoietic growth factor-induced proliferation of haematopoietic cells was also reported for anandamide [180]. Finally, 'endocannabinoids' may also act by modulating NO release from macrophages via activation and inhibition, respectively, of constitutive and inducible NO synthase [50,53].

In the brain, an astrocyte-mediated down-modulatory action on Theiler's virus or endotoxin-induced NO and TNF-α formation has been found for anandamide [181]. Indeed, the production of NAEs, including anandamide, and/or NaPEs has been associated in the past with noxious stimuli such as heart and cerebral ischaemia [19], neuronal injury [152], post-mortem brain damage [69–71], and very recently with haemorrhagic shock [182]. In the latter case, blood macrophages were suggested to be the cellular source of an hypotensive 'endocannabinoid', very probably anandamide. Therefore, it is possible that leukocytes recruited from blood following tissue damage may be responsible for the production of 'endocannabinoids' with vasodilatory and depressor activity (see above) or neuro- and cytoprotective effects [21]. Alternatively, much in the same way as their circulating homologues, populations of immunocompetent cells resident in brain and peripheral tissues may synthesise cytoprotective, anti-inflammatory 'endocannabinoids' during pathological responses.

### 5.6. Reproductive system

Tissues of the reproductive system have been shown to both contain CB1 receptors and synthesise as well as degrade anandamide. In rat testes and mouse vas deferens [67,165], this 'anandamidergic' system may be involved in the control of spermatogenesis and male fertility. Anandamide has been shown to inhibit the acrosome reaction through cannabinoid receptors located on sea urchin sperm cells [183,184], and sea urchin eggs have been found to synthesise and degrade anandamide [93], possibly with the purpose of inhibiting polyspermic fertilisation. The existence of an analogous mechanism in mammals has not been fully investigated yet. CB1 receptors have been found in mouse uterus, which also contain an 'anandamide synthase' distinct from FAAH whose levels appear to vary within the embryo implantation and inter-implantation sites [96,185]. The ECS was suggested to mediate the

chemical communication between the uterus and the embryo, as indicated by the presence of CB1 and CB2 receptors in embryos from very early stages of their development (from the one-cell to the blastocyst stages [186]). The synthesis of anandamide may be used by the uterus in order to direct the timing and placing of embryo implantation as suggested by the inhibitory effect of anandamide on embryonic cell division and implantation, as well as by the higher levels of 'anandamide synthase' and anandamide detected when the uterus is least receptive to implantation [74,96].

### 5.7. Ontogenic and developmental studies

Finally, the potential importance of the ECS seems to be emphasised by studies showing the presence of cannabinoid receptors and FAAH in pre- and postnatal brain [187,191]. The receptor tissue distribution [187], as well as studies on the response to prenatal exposure to anandamide [188], seems to suggest a function for the 'endocannabinoids' different from that played in adult mammals. No accurate data are available yet on the levels and tissue distribution of anandamide and 2-arachidonoylglycerol in foetal brain.

## 6. Future developments

Five years after the isolation of the first 'endocannabinoid', much progress has been made towards the understanding of the pharmacological actions and metabolic pathways of endogenous cannabimimetic fatty acids, also thanks to pre-existing knowledge on cannabinoid pharmacology [1,8,9] and NAE metabolism [19]. Apart from these findings, the importance of the ECS seems to be highlighted also by the discovery that cannabimimetic metabolites and cannabinoid receptors are widespread in the animal kingdom, and have been retained for over 500 million years in organisms as primitive as the leech, sea urchins and marine molluscs [50–52,93,189]. However, little is yet known on the molecular mechanisms underlying the regulation of 'endocannabinoid' metabolism as well as their correlation with physiopathological responses. Studies in this direction are to be encouraged if the development of ECS-related new drugs is to be eventually undertaken, as prompted by the centuries-old history of therapeutic exploitation of *Cannabis sativa*. Also our knowledge of basic animal physiology will be improved by these studies, which will require an ever closer co-operation among scientists from disciplines as far apart as chemistry, cell biology and psychology.

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## References

[1] R. Mechoulam, in: R. Mechoulam (Ed.), Cannabinoids as Therapeutic Agents, CRC Press, Boca Raton, 1986, pp. 1–20.
[2] Y. Gaoni, R. Mechoulam, J. Am. Chem. Soc. 86 (1964) 1646–1647.
[3] R.K. Razdan, in: J. Simon (Ed.), The Total Synthesis of Natural Products, Wiley, New York, 1981, pp. 185–262.
[4] W.L. Dewey, Pharmacol. Rev. 38 (1986) 151–178.
[5] A. Makriyannis, in: R. Pertwee (Ed.), Cannabinoid Receptors, Academic Press, London, 1995, pp. 87–115.
[6] W.A. Devane, F.A. Dysarz, M.R. Johnson, L.S. Melvin, A.C. Howlett, Mol. Pharmacol. 34 (1988) 605–613.
[7] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Science 258 (1992) 1946–1949.
[8] R.G. Pertwee, Pharmacol. Ther. 74 (1997) 129–180.
[9] A.C. Howlett, in: R. Pertwee (Ed.), Cannabinoid Receptors, Academic Press, London, 1995, pp. 167–204.
[10] B.R. Martin, Pharmacol. Rev. 38 (1986) 45–74.
[11] B.R. Martin, D.R. Compton, B.F. Thomas, W.R. Prescott, P.J. Little, R.K. Razdan, M.R. Johnson, L.S. Melvin, R. Mechoulam, S.J. Ward, Pharmacol. Biochem. Behav. 40 (1991) 471–478.
[12] E. Fride, R. Mechoulam, Eur. J. Pharmacol. 231 (1993) 313–314.
[13] J.N. Crawley, R.L. Corwin, J.K. Robinson, C.C. Felder, W.A. Devane, J. Axelrod, Pharmacol. Biochem. Behav. 46 (1993) 967–972.
[14] P.B. Smith, D.R. Compton, S.P. Welch, R.K. Razdan, R. Mechoulam, B.R. Martin, J. Pharmacol. Exp. Ther. 270 (1994) 219–227.

[15] V. Di Marzo, A. Fontana, Prostaglandins Leukotrienes Essent. Fatty Acids 53 (1995) 1–11.
[16] L. Hanus, A. Gopher, S. Almog, R. Mechoulam, J. Med. Chem. 36 (1993) 3032–3034.
[17] R. Pertwee, G. Griffin, L. Hanus, R. Mechoulam, Eur. J. Pharmacol. 259 (1994) 115–120.
[18] J. Barg, E. Fride, L. Hanus, R. Levy, N. Matus-Leibovitch, E. Heldman, M. Bayewitch, R. Mechoulam, Z. Vogel, Eur. J. Pharmacol. 287 (1995) 145–152.
[19] H.H.O. Schmid, P.C. Schmid, V. Natarajan, Prog. Lipid Res. 29 (1990) 1–43.
[20] L. Facci, R. Dal Toso, S. Romanello, A. Buriani, S.D. Skaper, A. Leon, Proc. Natl. Acad. Sci. USA 92 (1995) 3376–3380.
[21] S.D. Skaper, A. Buriani, R. Dal Toso, L. Petrelli, S. Romanello, L. Facci, A. Leon, Proc. Natl. Acad. Sci. USA 93 (1995) 3984–3989.
[22] T. Sheskin, L. Hanus, J. Slager, Z. Vogel, R. Mechoulam, J. Med. Chem. 40 (1997) 659–667.
[23] V.M. Showalter, D.R. Compton, B.R. Martin, M.E. Abood, J. Pharmacol. Exp. Ther. 278 (1996) 989–999.
[24] B.F. Cravatt, O. Prospero-Garcia, G. Siuzdak, N.B. Gilula, S.J. Henriksen, D.L. Boger, R.A. Lerner, Science 268 (1995) 1506–1509.
[25] D.L. Boring, B.A. Berglund, A.C. Howlett, Prostaglandins Leukotrienes Essent. Fatty Acids 55 (1996) 207–210.
[26] R. Mechoulam, E. Fride, L. Hanus, T. Sheskin, T. Bisogno, V. Di Marzo, M. Bayewitch, Z. Vogel, Nature 389 (1997) 25–26.
[27] J. Langstein, F. Hofstadter, H. Schwartz, Res. Immunol. 147 (1996) 389–396.
[28] R. Mechoulam, S. Ben-Shabat, L. Hanus, M. Ligumsky, N.E. Kaminski, A.R. Schatz, A. Gopher, S. Almog, B.R. Martin, D.R. Compton, R.G. Pertwee, G. Griffin, M. Bayewitch, J. Barg, Z. Vogel, Biochem. Pharmacol. 50 (1995) 83–90.
[29] T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, Biochem. Biophys. Res. Commun. 215 (1995) 89–97.
[30] M. Lee, K.H. Yang, N.E. Kaminski, J. Pharmacol. Exp. Ther. 275 (1995) 529–536.
[31] L.A. Matsuda, S.J. Lolait, M.J. Brownstein, A.C. Young, T.I. Bonner, Nature 346 (1990) 561–564.
[32] S. Munro, K.L. Thomas, M. Abu-Shaar, Nature 365 (1993) 61–65.
[33] M. Herkenham, in: R. Pertwee (Ed.), Cannabinoid Receptors, Academic Press, London, 1995, pp. 145–166.
[34] D. Shire, C. Carillon, M. Kaghad, B. Calandra, M. Rinaldi-Carmona, G. Le Fur, D. Caput, P. Ferrara, J. Biol. Chem. 270 (1995) 3726–3731.
[35] L. Vogel, J. Barg, R. Levy, D. Saya, E. Heldman, R. Mechoulam, J. Neurochem. 61 (1993) 352–355.
[36] C.C. Felder, E.M. Briley, J. Axelrod, J.T. Simpson, K. Mackie, W.A. Devane, Proc. Natl. Acad. Sci. USA 90 (1993) 7656–7660.
[37] M. Glass, C.C. Felder, J. Neurosci. 17 (1997) 5327–5333.
[38] E. Fride, J. Barg, R. Levy, D. Saya, E. Heldman, R. Mechoulam, Z. Vogel, J. Pharmacol. Exp. Ther. 272 (1995) 699–707.
[39] E. Shohami, J. Weidenfeld, H. Ovadia, Z. Vogel, L. Hanus, E. Fride, A. Breuer, S. Ben-Shabat, T. Sheskin, R. Mechoulam, CNS Drug Rev. 4 (1997) 429–451.
[40] K. Mackie, W.A. Devane, B. Hille, Mol. Pharmacol. 44 (1993) 498–503.
[41] K. Mackie, Y. Lai, R. Westenbroek, R. Mitchell, J. Neurosci. 15 (1995) 6552–6561.
[42] C.C. Felder, K.E. Joyce, E.M. Briley, J. Mansouri, K. Mackie, O. Blond, Y. Lai, A.L. Ma, R.L. Mitchell, Mol. Pharmacol. 48 (1995) 443–450.
[43] W. Twitchell, S. Brown, K. Mackie, J. Neurophysiol. 78 (1997) 43–50.
[44] T. Sugiura, T. Kodaka, S. Kondo, S. Nakane, H. Kondo, K. Waku, Y. Ishima, K. Watanabe, I. Yamamoto, J. Biochem. 122 (1997) 890–895.
[45] T. Sugiura, T. Kodaka, S. Kondo, T. Tonegawa, S. Nakane, S. Kishimoto, A. Yamashita, K. Waku, Biochem. Biophys. Res. Commun. 233 (1997) 207–210.
[46] M. Bouaboula, C. Poinot-Chazel, J. Marchand, X. Canat, B. Bourrie, M. Rinaldi-Carmona, B. Calandra, G. Le Fur, P. Casellas, Eur. J. Biochem. 237 (1996) 704–711.
[47] M. Wartmann, D. Campbell, A. Subramanian, S.H. Burstein, R.J. Davis, FEBS Lett. 359 (1995) 133–136.
[48] M. Rinaldi-Carmona, F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maurani, G. Neliat, D. Caput, P. Ferrara, P. Soubrié, J.C. Breliere, G. Le Fur, FEBS Lett. 350 (1994) 240–244.
[49] P. Derkinderen, M. Toutant, F. Burgaya, M. Le Bert, J.C. Siciliano, V. de Franciscis, M. Gelman, J.-A. Girault, Science 273 (1996) 1719–1722.
[50] G.B. Stefano, Y. Liu, M.S. Goligorsky, J. Biol. Chem. 271 (1996) 19238–19242.
[51] G.B. Stefano, B. Salzet, M. Salzet, Brain Res. 753 (1997) 219–224.
[52] G.B. Stefano, B. Salzet, C.M. Rialas, M. Pope, A. Kustka, K. Neenan, S. Pryor, M. Salzet, Brain Res. 763 (1997) 63–68.
[53] R.G. Coffey, Y. Yamamoto, E. Snella, S. Pross, Biochem. Pharmacol. 52 (1996) 743–751.
[54] L. De Petrocellis, D. Melck, A. Palmisano, T. Bisogno, C. Laezza, M. Bifulco, V. Di Marzo, Proc. Natl. Acad. Sci. USA (1998) in press.
[55] A.C. Shivachar, B.R. Martin, E.F. Ellis, Biochem. Pharmacol. 51 (1996) 669–676.
[56] V. Di Marzo, L. De Petrocellis, T. Bisogno, S. Maurelli, Adv. Exp. Med. Biol. 407 (1997) 341–346.
[57] S.A. Hunter, S.H. Burstein, Life Sci. 60 (1997) 1563–1573.
[58] L. Venance, D. Piomelli, J. Glowinski, C. Giaume, Nature 376 (1995) 590–594.
[59] L. Venance, S. Sagan, C. Giaume, Pflugers Arch. Eur. J. Physiol. 434 (1997) 147–149.
[60] D.E. Johnson, S.L. Heald, R.D. Dally, R.A. Janis, Prostaglandins Leukotrienes Essent. Fatty Acids 48 (1993) 429–437.

[61] K. Shimasue, T. Urushidani, M. Hagiwara, T. Nagao, Eur. J. Pharmacol. 296 (1996) 347–350.
[62] J.S. Poling, M.A. Rogawski, N. Salem, S. Vicini, Neuropharmacology 35 (1996) 983–991.
[63] P. Fan, J. Neurophysiol. 73 (1995) 907–910.
[64] L. De Petrocellis, P. Orlando, V. Di Marzo, Biochem. Mol. Biol. Int. 36 (1995) 1127–1133.
[65] A.J. Hampson, L.M. Bornheim, M. Scanziani, C.S. Yost, A.T. Gray, B.M. Hansen, D.J. Leonoudakjs, P.E. Bickler, J. Neurochem. 70 (1998) 671–676.
[66] D. Koga, T. Santa, K. Hagiwara, K. Imai, H. Takizawa, T. Nagano, M. Hirobe, M. Ogawa, T. Sato, K. Inoue, I. Kudo, Biomed. Chromatogr. 9 (1995) 56–57.
[67] T. Sugiura, S. Kondo, A. Sukagawa, T. Tonegawa, S. Nakane, A. Yamashita, K. Waku, Biochem. Biophys. Res. Commun. 218 (1996) 113–117.
[68] A. Fontana, V. Di Marzo, H. Cadas, D. Piomelli, Prostaglandins Leukotrienes Essent. Fatty Acids 53 (1995) 301–308.
[69] P.C. Schmid, R.J. Krebsbach, S.R. Perry, T.M. Dettmer, J.L. Maassen, H.H.O. Schmid, FEBS Lett. 375 (1995) 117–120 plus 385 (1996) 125–126 (correction).
[70] K. Kempe, F.F. Hsu, A. Bohrer, J. Turk, J. Biol. Chem. 271 (1996) 17287–17295.
[71] C.C. Felder, A. Nielsen, E.M. Briley, M. Palkovitis, J. Priller, J. Axelrod, D.N. Nguyen, J.M. Richardson, R.M. Riggin, G.A. Koppel, S.M. Paul, G.W. Becker, FEBS Lett. 393 (1996) 231–235.
[72] D. Koga, T. Santa, T. Fukushima, H. Homma, K. Imai, J. Chromatogr. 690 (1997) 7–13.
[73] D.G. Deutsch, M.S. Goligorsky, P.C. Schmid, R.J. Krebsbach, H.H.O. Schmid, S.K. Das, S.K. Dey, G. Arreaza, C. Thorup, G.B. Stefano, L.C. Moore, J. Clin. Invest. 100 (1997) 1538–1546.
[74] P.C. Schmid, B.C. Paria, R.J. Krebsbach, H.H.O. Schmid, S.K. Dey, Proc. Natl. Acad. Sci. USA 94 (1997) 4188–4192.
[75] T. Bisogno, S. Maurelli, D. Melck, L. De Petrocellis, V. Di Marzo, J. Biol. Chem. 272 (1997) 3315–3323.
[76] P.C. Schmid, T. Kuwae, R.J. Krebsbach, H.H.O. Schmid, Chem. Phys. Lipids 87 (1997) 103–110.
[77] T. Bisogno, K. Katayama, D. Melck, N. Ueda, L. De Petrocellis, S. Yamamoto, V. Di Marzo, Eur. J. Biochem. (1998) in press.
[78] N. Stella, P. Schweitzer, D. Piomelli, Nature 388 (1997) 773–778.
[79] S. Ben-Shabat, E. Fride, T. Sheskin, T. Tamiri, M.-H. Rhee, Z. Vogel, T. Bisogno, L. De Petrocellis, V. Di Marzo, R. Mechoulam, Eur. J. Pharmacol. (1998) in press.
[80] T. Bisogno, N. Sepe, L. De Petrocellis, R. Mechoulam, V. Di Marzo, Biochem. Biophys. Res. Commun. 239 (1997) 473–479.
[81] K.K. Kruszka, R.W. Gross, J. Biol. Chem. 269 (1994) 14345–14348.
[82] W.A. Devane, J. Axelrod, Proc. Natl. Acad. Sci. USA 91 (1994) 6698–6701.
[83] N. Ueda, Y. Kurahashi, S. Yamamoto, T. Tokunaga, J. Biol. Chem. 270 (1995) 23823–23827.
[84] Y. Kurahashi, N. Ueda, H. Suzuki, M. Suzuki, S. Yamamoto, Biochem. Biophys. Res. Commun. 237 (1997) 512–515.
[85] G. Arreaza, W.A. Devane, R.L. Omeir, G. Sajnani, J. Kunz, B.F. Cravatt, D.G. Deutsch, Neurosci. Lett. 234 (1997) 59–62.
[86] V. Di Marzo, A. Fontana, H. Cadas, S. Schinelli, G. Cimino, J.-C. Schwartz, D. Piomelli, Nature 372 (1994) 686–691.
[87] T. Sugiura, S. Kondo, A. Sukagawa, T. Tonegawa, S. Nakane, A. Yamashita, Y. Ishima, K. Waku, Eur. J. Biochem. 240 (1996) 53–62.
[88] P.C. Schmid, P.V. Reddy, V. Natarajan, H.H.O. Schmid, J. Biol. Chem. 258 (1983) 9302–9306.
[89] V. Di Marzo, L. De Petrocellis, T. Sugiura, K. Waku, Biochem. Biophys. Res. Commun. 227 (1996) 281–288.
[90] H. Cadas, E. di Tomaso, D. Piomelli, J. Neurosci. 17 (1997) 1226–1242.
[91] H. Cadas, S. Gaillet, M. Beltramo, L. Venance, D. Piomelli, J. Neurosci. 16 (1996) 3934–3942.
[92] V. Di Marzo, L. De Petrocellis, N. Sepe, A. Buono, Biochem. J. 316 (1996) 977–984.
[93] T. Bisogno, M. Ventriglia, M. Mosca, A. Milone, G. Cimino, V. Di Marzo, Biochim. Biophys. Acta 1345 (1997) 338–348.
[94] M.E. Blank, E.A. Cress, M. Robinson, F. Snyder, Biochim. Biophys. Acta 833 (1985) 366–371.
[95] T. Kuwae, P.C. Schmid, H.H.O. Schmid, Biochim. Biophys. Acta 1344 (1997) 74–86.
[96] B.C. Paria, D.G. Deutsch, S.K. Dey, Mol. Reprod. Dev. 45 (1996) 183–192.
[97] T. Bisogno, N. Sepe, D. Melck, S. Maurelli, L. De Petrocellis, V. Di Marzo, Biochem. J. 322 (1997) 671–677.
[98] V. Di Marzo, L. De Petrocellis, T. Bisogno, D. Melck, N. Sepe, in: R.A. Riemersma, R. Wilson (Eds.), Proceedings of the 4th International Congress on Essential Fatty Acids and Eicosanoids AOCS Press, 1998, in press.
[99] J. Meldolesi, E.W. Westhead, in: R.H. Michell, A.H. Drummond, C.P. Downes (Eds.), Inositol Lipids in Cell Signalling, Academic Press, London, 1989, pp. 31–336.
[100] V. Di Marzo, Prostaglandins Leukotrienes Essent. Fatty Acids 53 (1995) 239–254.
[101] T. Sugiura, S. Kondo, T. Kodaka, T. Tonegawa, S. Nakane, A. Yamashita, Y. Ishima, K. Waku, Biochem. Mol. Biol. Int. 40 (1996) 931–938.
[102] D.J. Merkler, K.A. Merkler, W. Stern, F.F. Fleming, Arch. Biochem. Biophys. 330 (1996) 430–434.
[103] D.J. Merkler, K.A. Merkler, Proceedings of the 5th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases, La Jolla, September 17–20, 1997, p. 42.
[104] C.J. Hillard, W.S. Edgemond, A. Jarrahian, W.B. Campbell, J. Neurochem. 68 (1997) 631–638.
[105] M. Beltramo, N. Stella, A. Calignano, S.Y. Lin, A. Makriyannis, D. Piomelli, Science 277 (1997) 1094–1097.

[106] V. Di Marzo, T. Bisogno, T. Sugiura, D. Melck, L. De Petrocellis, Biochem. J. 331 (1998) 15–19.
[107] D.G. Deutsch, S.A. Chin, Biochem. Pharmacol. 46 (1993) 791–796.
[108] F. Desarnaud, H. Cadas, D. Piomelli, J. Biol. Chem. 270 (1995) 6030–6035.
[109] C.J. Hillard, D.M. Wilkison, W.S. Edgemond, W.B. Campbell, Biochim. Biophys. Acta 1257 (1995) 249–256.
[110] K. Watanabe, Y. Kayano, T. Matsunaga, I. Yamamoto, H. Yoshimura, Biol. Pharmaceut. Bull. 19 (1997) 1109–1111.
[111] B. Koutek, G.D. Prestwich, A.C. Howlett, S.A. Chin, D. Salehani, N. Akhavan, D.G. Deutsch, J. Biol. Chem. 269 (1994) 22937–22940.
[112] P.C. Schmid, M.L. Zuzarte-Augustin, H.H.O. Schmid, J. Biol. Chem. 260 (1985) 14145–14149.
[113] B.F. Cravatt, D.K. Giang, S.P. Mayfield, D.L. Boger, R.A. Lerner, N.B. Gilula, Nature 384 (1996) 83–87.
[114] S. Maurelli, T. Bisogno, L. De Petrocellis, A. Di Luccia, G. Marino, V. Di Marzo, FEBS Lett. 377 (1995) 82–86.
[115] D.K. Giang, B.F. Cravatt, Proc. Natl. Acad. Sci. USA 94 (1997) 2238–2242.
[116] K. Katayama, N. Ueda, Y. Kurahashi, H. Suzuki, S. Yamamoto, I. Kato, Biochim. Biophys. Acta 1347 (1997) 212–218.
[117] S. Matsuda, N. Kanemitsu, A. Nakamura, Y. Mimura, N. Ueda, Y. Kurahashi, S. Yamamoto, Exp. Eye Res. 64 (1997) 707–711.
[118] S.K. Goparaju, N. Ueda, H. Yamaguchi, S. Yamamoto, FEBS Lett. 422 (1998) 69–73.
[119] L. De Petrocellis, D. Melck, N. Ueda, S. Maurelli, Y. Kurahashi, S. Yamamoto, G. Marino, V. Di Marzo, Biochem. Biophys. Res. Commun. 231 (1997) 82–88.
[120] D.G. Deutsch, R. Omeir, G. Arreaza, D. Salehani, G.D. Prestwich, Z. Huang, A.C. Howlett, Biochem. Pharmacol. 53 (1997) 255–260.
[121] J.E. Patterson, I.R. Ollman, B.F. Cravatt, D.L. Boger, G.-H. Wong, R.A. Lerner, J. Am. Chem. Soc. 118 (1996) 5938–5945.
[122] D.G. Deutsch, S. Lin, W.A.G. Hill, K.L. Morse, D. Salehani, G. Arreaza, R.L. Omeir, A. Makriyannis, Biochem. Biophys. Res. Commun. 231 (1997) 217–221.
[123] M. Beltramo, E. di Tomaso, D. Piomelli, FEBS Lett. 403 (1997) 263–267.
[124] S.L. Hazen, L.A. Zupan, R.H. Weiss, D.P. Getman, R.W. Gross, J. Biol. Chem. 266 (1991) 7227–7232.
[125] C.J. Fowler, G. Tiger, A. Stenstrom, J. Pharmacol. Exp. Ther. 283 (1997) 729–734.
[126] L. De Petrocellis, D. Melck, N. Ueda, Y. Kurahashi, T. Bisogno, S. Yamamoto, V. Di Marzo, Adv. Exp. Med. Biol. 433 (1998) 259–263.
[127] S.R. Fernando, R.G. Pertwee, Br. J. Pharmacol. 121 (1997) 1716–1720.
[128] L.M. Bornheim, K.Y. Kim, B. Chen, M.A. Correia, Biochem. Biophys. Res. Commun. 197 (1993) 740–746.
[129] N. Ueda, K. Yamamoto, S. Yamamoto, T. Tokunaga, E. Shirakawa, H. Shinaki, M. Ogawa, T. Sato, I. Kudo, K. Inoue, H. Takizawa, T. Nagano, M. Hirobe, H. Saito, Biochim. Biophys. Acta 1254 (1995) 127–134.
[130] A.J. Hampson, W.A. Hill, M. Zan-Phillips, A. Makriyannis, E. Leung, R.M. Eglen, L.M. Bornheim, Biochim. Biophys. Acta 1259 (1995) 173–179.
[131] W.S. Edgemond, C.J. Hillard, J.R. Falck, C.S. Kearn, W.B. Campbell, Proc. Int. Cann. Res. Soc. 1997 Meeting, p. 38.
[132] M. Yu, D. Ives, S. Ramesha, J. Biol. Chem. 272 (1997) 21181–21186.
[133] M.L. Wise, K. Soderstrom, T.F. Murray, W.H. Gerwick, Experientia 52 (1996) 88–92.
[134] M. van der Stelt, A.M. Paoletti, M. Maccarrone, W.F. Nieuwenhuizen, G. Bagetta, G.A. Veldink, A. Finazzi Agrò, J.F. Vliegenthart, FEBS Lett. 415 (1997) 313–316.
[135] E. di Tomaso, M. Beltramo, D. Piomelli, Nature 382 (1997) 677–678.
[136] E. Fride, T. Bisogno, V. Di Marzo, M. Bayewitch, Z. Vogel, R. Mechoulam, Soc. Neurosci. Abstr. 23 (1997) 486.3.
[137] K.A. Willoughby, S.F. Moore, B.R. Martin, E.F. Ellis, J. Pharmacol. Exp. Ther. 282 (1997) 243–247.
[138] V. Abadji, S. Lin, G. Taha, G. Griffin, L.A. Stevenson, R.G. Pertwee, A. Makriyannis, J. Med. Chem. 37 (1994) 1889–1893.
[139] V. Di Marzo, L. De Petrocellis, T. Bisogno, S. Maurelli, J. Drug Dev. Clin. Pract. 7 (1995) 199–219.
[140] A.-K. Cadogan, S.P.H. Alexander, E.A. Boyd, D.A. Kendall, J. Neurochem. 69 (1997) 1131–1137.
[141] J. Romero, L. Garcia, M. Cebeira, D. Zadrozny, J.J. Fernandez-Ruiz, J.A. Ramos, Life Sci. 56 (1995) 2033–2040.
[142] J. Romero, E. Garcia-Palomero, J.J. Fernandez-Ruiz, J.A. Ramos, Behav. Pharmacol. 7 (1996) 299–302.
[143] A.P. Wickens, R.G. Pertwee, Eur. J. Pharmacol. 250 (1993) 205–208.
[144] J.J. Anderson, A.M. Kask, T.N. Chase, Eur. J. Pharmacol. 295 (1996) 163–168.
[145] P.E. Mallet, R.J. Beninger, Behav. Pharmacol. 7 (1996) 276–284.
[146] C. Castellano, S. Cabib, A. Palmisano, V. Di Marzo, S. Puglisi-Allegra, Behav. Pharmacol. 8 (1997) 707–712.
[147] J.-P. Terranova, J.-J. Storme, N. Lafon, A. Perio, M. Rinaldi-Carmona, G. Le Fur, P. Soubrié, Psychopharmacology 126 (1996) 165–172.
[148] A.N. Gifford, C.R. Ashby Jr., J. Pharmacol. Exp. Ther. 277 (1996) 1431–1436.
[149] J.P. Terranova, J.C. Michaud, G. Lefur, P. Soubrié, Naunyn Schmiedeberg's Arch. Pharmacol. 352 (1995) 576–579.
[150] C. Collin, W.A. Devane, D. Dahl, C.-C. Lee, J. Axelrod, D.L. Alkon, Proc. Natl. Acad. Sci. USA 92 (1995) 10167–10171.
[151] M. Shen, T.M. Piser, V.S. Seybold, S.A. Thayer, J. Neurosci. 16 (1996) 4322–4334.
[152] H.S. Hansen, L. Lauritzen, A.M. Strand, A.M. Vinggaard, A. Frandsen, A. Schousboe, J. Neurochem. 69 (1997) 753–761.
[153] J. Weidenfeld, S. Feldman, R. Mechoulam, Neuroendocrinology 59 (1994) 110–112.

[154] T. Wenger, B.E. Toth, B.R. Martin, Life Sci. 56 (1995) 2057–2063.
[155] J. Romero, L. Garcia, J.A. Ramos, J.J. Fernandez-Ruiz, Neuroendocrinol. Lett. 16 (1994) 159–164.
[156] J.J. Fernandez-Ruiz, R.M. Munoz, J. Romero, M.A. Villanua, A. Makriyannis, J. Ramos, Biochem. Pharmacol. 53 (1997) 1919–1927.
[157] T. Wenger, K.A. Jamali, C. Juaneda, J. Leonardelli, G. Tramu, Biochem. Biophys. Res. Commun. 273 (1997) 724–728.
[158] V. Santucci, J.J. Storme, P. Soubrie, G. Le Fur, Life Sci. 58 (1996) 103–110.
[159] G. Pugh, P.B. Smith, D.S. Dombrowski, S.P. Welch, J. Pharmacol. Exp. Ther. 279 (1996) 608–616.
[160] S.P. Welch, L.D. Dunlow, G.S. Patrick, R.K. Razdan, J. Pharmacol. Exp. Ther. 273 (1995) 1235–1244.
[161] J.D. Richardson, L. Aanonsen, K.M. Hargreaves, J. Neurosci. 18 (1998) 451–457.
[162] A.G. Hohmann, M.B. Whiteside, M. Herkenham, Proc. Int. Cann. Res. Soc. 1997 Meeting, p. 60.
[163] R.G. Pertwee, S.R. Fernando, J.E. Nash, A.A. Coutts, Br. J. Pharmacol. 118 (1996) 2199–2205.
[164] R.G. Pertwee, S.R. Fernando, Br. J. Pharmacol. 118 (1996) 2053–2058.
[165] R.G. Pertwee, G. Joeadigwe, G.M. Hawksworth, Eur. J. Pharmacol. 296 (1996) 169–172.
[166] K. Varga, K. Lake, B.R. Martin, G. Kunos, Eur. J. Pharmacol. 278 (1995) 279–283.
[167] K. Varga, K. Lake, D.H. Huang-fu, P.G. Guyenet, G. Kunos, Hypertension 28 (1996) 682–686.
[168] E.J.N. Ishac, L. Jiang, K.D. Lake, K. Varga, M.E. Abood, G. Kunos, Br. J. Pharmacol. 118 (1996) 2023–2028.
[169] J.-V. Mombouli, P.M. Vanhoutte, Trends Pharmacol. Sci. 18 (1997) 252–256.
[170] M.D. Randall, S.P.H. Alexander, T. Bennett, E.A. Boyd, J.R. Fry, S.M. Gardiner, P.A. Kemp, A.I. McCulloch, D.A. Kendall, Biochem. Biophys. Res. Commun. 229 (1996) 114–120.
[171] M.D. Randall, A.I. McCulloch, D.A. Kendall, Eur. J. Pharmacol. 333 (1997) 191–197.
[172] F. Plane, M. Holland, G.J. Waldron, C.J. Garland, J.P. Boyle, Br. J. Pharmacol. 121 (1997) 1509–1511.
[173] D.W. Pate, K. Jarvinen, A. Urtti, P. Jarho, T. Jarvinen, Curr. Eye Res. 14 (1995) 791–797.
[174] Y. Mikawa, S. Matsuda, T. Kanagawa, T. Tajika, N. Ueda, Y. Mimura, Jpn. J. Ophthalmol. 41 (1997) 217–220.
[175] S. Mazzari, R. Canella, L. Petrelli, G. Marcolongo, A. Leon, Eur. J. Pharmacol. 300 (1996) 227–236.
[176] E.V. Berdyshev, E. Boichot, N. Germain, N. Allain, J.-P. Anger, V. Lagente, Eur. J. Pharmacol. 330 (1997) 231–240.
[177] H. Schwarz, F.J. Blanco, M. Lotz, J. Neuroimmunol. 55 (1994) 107–115.
[178] M. Bayewitch, T. Avidor-Reiss, R. Levy, J. Barg, R. Mechoulam, Z. Vogel, FEBS Lett. 375 (1995) 143–147.
[179] G.A. Cabral, D.M. Toney, K. Fischer-Stenger, M.P. Harrison, F. Marciano-Cabral, Life Sci. 56 (1995) 2065–2072.
[180] P. Valk, S. Verbakel, Y. Vankan, S. Hol, S. Mancham, R. Ploemacher, A. Mayen, B. Lowenberg, R. Delwel, Blood 90 (1997) 1448–1457.
[181] F. Molina Holgado, A. Lledo, C. Guaza, NeuroReport 8 (1997) 1929–1933.
[182] J.A. Wagner, K. Varga, E.F. Ellis, B.A. Rzigalinki, B.R. Martin, G. Kunos, Nature 390 (1997) 518–521.
[183] M.C. Chang, D. Berkery, R. Schuel, S.G. Laychock, A.M. Zimmerman, S. Zimmerman, H. Schuel, Mol. Reprod. Dev. 36 (1993) 507–516.
[184] H. Schuel, E. Goldstein, R. Mechoulam, A.M. Zimmerman, S. Zimmerman, Proc. Natl. Acad. Sci. USA 91 (1994) 7678–7682.
[185] S.K. Das, B.C. Paria, I. Chakraborty, S.K. Dey, Proc. Natl. Acad. Sci. USA 92 (1995) 4332–4336.
[186] B.C. Paria, S.K. Das, S.K. Dey, Proc. Natl. Acad. Sci. USA 92 (1995) 9460–9464.
[187] E. Fride, R. Mechoulam, Psychoneuroendocrinology 21 (1996) 157–172.
[188] J. Romero, E. Garcia-Palomero, F. Berrendero, L. Garcia-Gil, M.L. Hernandez, J.A. Ramos, J.J. Fernandez-Ruiz, Synapse 26 (1997) 317–323.
[189] N. Sepe, L. De Petrocellis, F. Montanaro, G. Cimino, V. Di Marzo, Biochim. Biophys. Acta 1389 (1998) 101–111.
[190] X. Guan, B.F. Cravatt, G.R. Ehring, J.E. Hall, D.L. Boger, R.A. Lerner, N.B. Gilula, J. Cell Biol. 139 (1997) 1785–1792.
[191] E.A. Thomas, B.F. Cravatt, P.E. Danielson, N.B. Gilula, J.G. Sutcliffe, J. Neurosci. Res. 50 (1997) 1047–1052.
[192] J.L. Wiley, K.M. Golden, W.J. Ryan, R.L. Balster, R.K. Razdan, B.R. Martin, Pharmacol. Biochem. Behav. 58 (1997) 1139–1143.
[193] A. Jarrahian, C.J. Hillard, Prostaglandins Leukotrienes Essent. Fatty Acids 57 (1997) 551–554.
[194] R. White, C.R. Hiley, Br. J. Pharmacol. 122 (1997) 1573–1584.
[195] P.M. Zygmunt, E.D. Hogestatt, K. Waldeck, G. Edwards, A.J. Kirkup, A.H. Weston, Br. J. Pharmacol. 122 (1997) 1679–1686.
[196] P.F. Pratt, C.J. Hillard, W.S. Edgemond, W.B. Campbell, Am. J. Physiol. 274 (1998) H375–H381.
[197] T. Sugiura, T. Kodaka, S. Nakane, S. Kishimoto, S. Kondo, K. Waku, Biochem. Biophys. Res. Commun. 243 (1998) 838–843.
[198] D.W. Pate, K. Jarvinen, A. Urtti, P. Jarho, V. Mahadevan, T. Jarvinen, Pharmacol. Res. 14 (1997) 1738–1743.
[199] C.C. Felder, K.E. Joyce, E.M. Briley, M. Glass, K. Mackie, K.J. Fahey, G.J. Cullinan, D.C. Hunden, D.W. Johnson, M.O. Chaney, G.A. Koppel, M. Brownstein, J. Pharmacol. Exp. Ther. 284 (1988) 291–297.
[200] E. Sulcova, R. Mechoulam, E. Fride, Pharmacol. Biochem. Behav. 59 (1988) 347–352.
[201] J.D. Richardson, S. Kilo, K.M. Hargreaves, Pain 75 (1998) 111–119.