Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action

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The existence of an endogenous cannabinoid system was demonstrated conclusively with the discovery of endogenous brain constituents capable of activating the cannabinoid receptors functionally. These compounds are synthesized by neuronal cells and inactivated through re-uptake and enzymatic hydrolysis by both neurons and astrocytes. In analogy with the endorphins they can be referred to as endocannabinoids. Apart from the identification of their metabolic pathways, research carried out in the past six years has focused on the possible cellular and molecular targets for the actions of endocannabinoids. These studies have confirmed a similarity between the endocannabinoids and the psychoactive substance in marijuana, Δ^9(–)-tetrahydrocannabinol, and have suggested a role for endocannabinoids in the modulation of neurotransmitter action and release.

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THE THERAPEUTIC AND RECREATIONAL USE of marijuana has been practised for several centuries[1], although intensive research on the molecular mode of action of *Cannabis sativa* preparations started only in the 1960s, when the chemical structure of marijuana's principle psychoactive ingredient, Δ^9(–)-tetrahydrocannabinol (THC), was characterized[2]. Owing to the lipophilicity and cell membrane-perturbating action of THC (Ref. 3), it was not thought that this compound acted through specific receptors. However, several pharmacologists became convinced of the existence of specific membrane binding sites for this compound when, in 1988, the synthesis of a high-affinity cannabinoid ligand allowed the characterization of cannabinoid receptors[4]. After the cloning and molecular characterization of the first cannabinoid-receptor subtype[5] (previously known as central subtype, this was later named CB_1 following the discovery of the CB_2 subtype[6], which was originally known as the peripheral subtype) and the mapping of its distribution in the brain[7], researchers have embraced the idea that cannabinoid receptors do exist and mediate most of the effects of THC and marijuana in central and peripheral tissues. Moreover, it has also been confirmed that cannabinoid receptors belong to the seven transmembrane domain family of G-protein-coupled receptors. However, one might wonder why brain regions such as the cortex, basal ganglia, cerebellum and hippocampus should synthesize high amounts of receptors for a substance whose only natural source is a plant with no strict biological link with mammals. Are there endogenous ligands selective for cannabinoid receptors, whose action is mimicked by THC?

Endocannabinoids and the endogenous cannabinoid system

In 1992, the first endogenous ligand of CB_1 receptors was identified in porcine brain[8]. This substance is the amide of arachidonic acid (AA) with ethanolamine, and was named anandamide from the Sanskrit word 'ananda' meaning 'bliss'. This brain component was able to reproduce the most typical behavioural effects of THC in rodents, that is, inhibition of locomotor activity in open-field and ring-immobility tests, analgesia on a hot plate, and rectal hypothermia[9]. Moreover, anandamide exhibited cross-tolerance to THC in these tests (although only at high concentrations)[10], substituted for THC in drug-discrimination tests[11], and shared with THC the same G-protein-mediated actions on adenylate cyclase and Ca^{2+} channels[9,12,13]. Anandamide, thus, seemed to possess the characteristics of an ideal modulator of neurotransmitter release or action, or both. Some differences between anandamide and exogenous cannabinoids were also observed. These included a low potency of anandamide in some *in vitro* assays, which suggested that anandamide might have an action as a partial CB_1-receptor agonist[13], and a weaker- and shorter-lasting action *in vivo* of the endogenous metabolite when compared with the most active synthetic compounds[9]. This latter phenomenon, however, is likely to be due to the recently reported rapid degradation of anandamide *in vivo*[14]. Despite the fact that the existence of other, possibly peptide-like, endogenous ligands of cannabinoid receptors has been suggested[9,15], the only other metabolites characterized in the brain, since the discovery of anandamide, that have been proved to behave as functional agonists of cannabinoid receptors have been polyunsaturated fatty acid derivatives which have no higher efficacy than anandamide in these assays of THC-like activity. These compounds include docosatetraenoyl-ethanolamide, di-homo-γ-linolenoyl-ethanolamide[16], and 2-arachidonoylglycerol (2-AG)[17,18], and, analagous to the endorphins, can be collectively termed endocannabinoids (Fig. 1).

Our understanding of the neurophysiological role of cannabinoid receptors and endocannabinoids (which together form the endogenous cannabinoid

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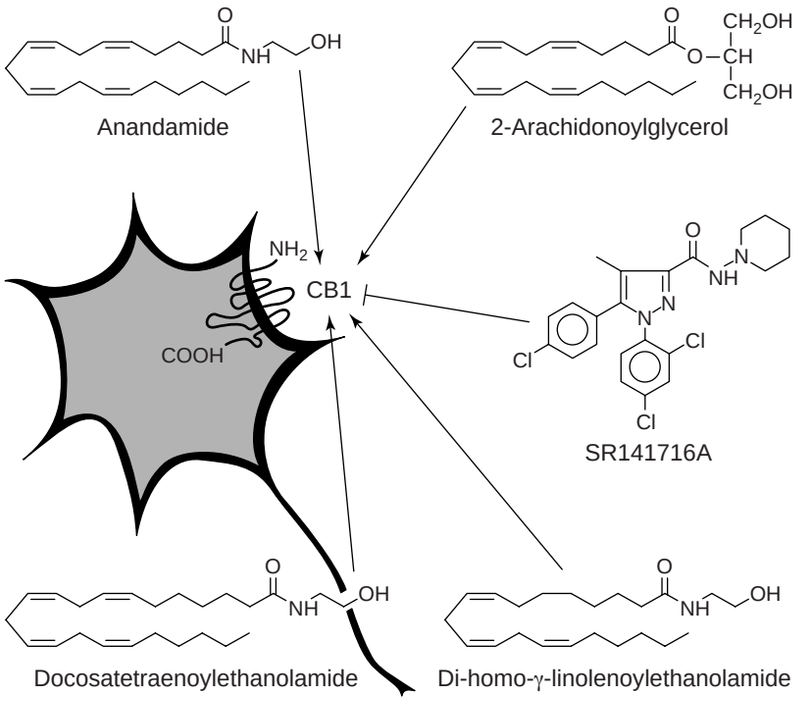


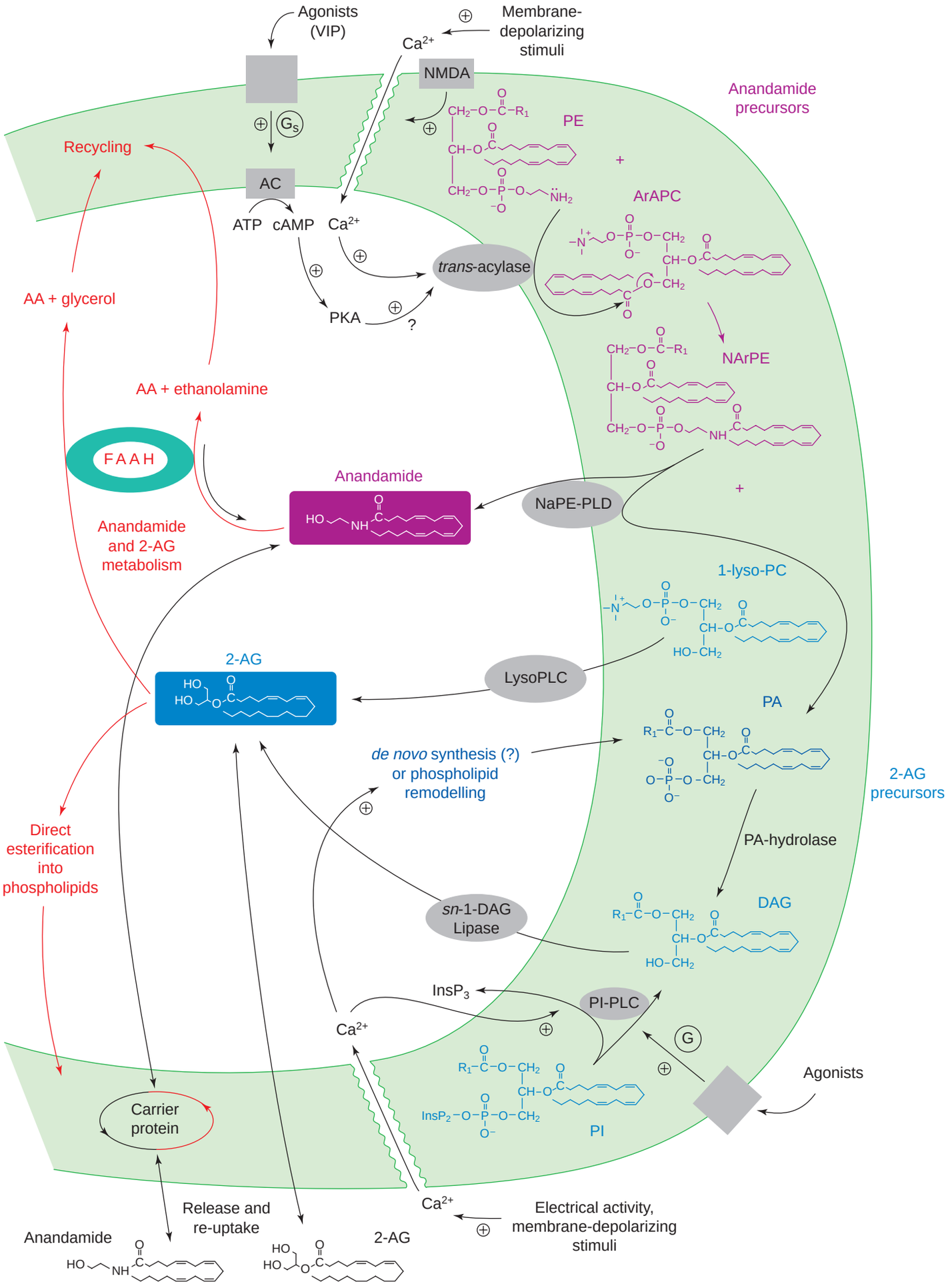
Fig. 1. ***Endogenous ligands of cannabinoid CB_1 receptors.*** *The cannabimimetic* N*-acylethanolamines were previously collectively referred to as anandamides[9], but the name anandamide is now used only for* N*-arachidonoylethanolamine, the most thoroughly studied endocannabinoid. Although reverse agonism has been shown for SR141716A (Ref. 19), this selective antagonist of CB_1 receptors[20] is used both* in vivo *and* in vitro *also as a tool to reveal a tonic control by endogenous cannabinoids of some physiological functions.*

system) benefits from knowledge acquired from more than three decades of research on cannabinoid pharmacology and cannabinoid receptor distribution and function[21]. This knowledge, however, needs to be supplemented with new data that deal specifically with endocannabinoid biosynthesis, action and inactivation. In particular, the distribution of cannabinoid receptors should be correlated with that of the enzymes involved in endocannabinoid metabolism. The contribution of the endogenous cannabinoid system to the control of nervous functions *in vivo* can then be assessed using cannabinoid-receptor antagonists, such as the CB_1-receptor-selective compound, SR141716A (Ref. 20, Fig. 1), and inhibitors of endocannabinoid metabolism, or by establishing cause–effect relationships between physiological events and changes in endocannabinoid levels. Data of this kind, particularly for anandamide, have become available during the past six years and allow us to draw some conclusions on the functional significance of the endocannabinoids in the nervous system.

How the brain makes and disposes of endocannabinoids

After the discovery of anandamide and its cannabimimetic properties, the first step towards establishing its possible neurophysiological role was to find mechanisms for its biosynthesis and inactivation in the CNS (Fig. 2). Studies carried out in the rat[22] showed that the synthesis and release of anandamide, whose basal levels in brain are low compared to most neurotransmitters[23], could be stimulated in intact cortical and striatal neurones (but not astrocytes) by treatment with membrane-depolarizing agents such as ionomycin, 4-aminopyridine, kainate and high K^+. Neurones and astrocytes were also both found to re-uptake and hydrolyse anandamide rapidly, resulting in the formation of AA and ethanolamine[22,36]. The uptake mechanism is mediated by a saturable, selective, temperature-dependent and Na^+-independent transporter[22,24,25] that also facilitates anandamide release[24], and whose inhibition can potentiate some of anandamide's actions[25]. Thus, anandamide, like classical neurotransmitters, is released from neurones following membrane depolarization and Ca^{2+} influx into the cell, and is inactivated through re-uptake and enzymatic degradation mechanisms. However, unlike classical neurotransmitters, anandamide is thought to be synthesized from the phospholipase D-catalysed hydrolysis of a phospholipid precursor, *N*-arachidonoyl-phosphatidylethanolamine (NArPE, Fig. 2)[22]. This biosynthetic pathway is identical to that proposed in the 1980s for the biosynthesis of saturated and mono-unsaturated *N*-acylethanolamines[26] (which are produced in higher amounts than anandamide by ionomycin-stimulated neurones), and could also apply to the other cannabimimetic fatty acid amides shown in Fig. 1. The other route proposed for the synthesis of anandamide is the ATP- and co-enzyme-A-independent condensation of high concentrations of AA and

Fig. 2. (facing page) ***Biosynthesis and inactivation of endocannabinoids.*** *Endocannabinoids are membrane-derived local neuromodulators that are synthesized on demand. They can, in principle, be released from both pre- and postsynaptic nerve terminals. The two enzymes necessary for the biosynthesis of anandamide and other* N*-acylethanolamines are a Ca^{2+}-dependent* trans*-acylase and an* N*-acyl-phosphatidylethanolamine-selective phospholipase D (NaPE-PLD), probably distinct from the PLD enzyme that catalyses phosphatidylethanolamine (PE) and phosphatidylcholine (PC) breakdown[22,26–30]. A by-product of the first reaction is 1-lyso-PC, which might serve, together with other 1-lyso-phospholipids[18], as a precursor for the one-step formation of 2-arachidonoylglycerol (2-AG)[30]. The latter compound could also be produced from lysophosphatidic acid and triglycerides (not shown). In most cases studied so far, however,* sn*-2-arachidonate-containing diacylglycerols (DAGs) serve as precursors for 2-AG through the action of a* sn*-1-selective DAG lipase[30–32]. In cortical neurones, DAGs are derived from phosphoinositides (PI) through the action of a PI-selective phospholipase C (PI-PLC)[32]. DAGs may also derive from phosphatidic acid (PA) obtained from PLC-independent pathways (shown in dark blue), such as* de novo *synthesis,* N*-acylethanolamine formation or phospholipid remodelling[18,30,31]. Once synthesized, anandamide is released from neurons through facilitated diffusion[24]. The same carrier probably also mediates anandamide re-uptake by neurones[22,24,25]. 2-Arachidonoylglycerol and, in part, anandamide are also capable of diffusing passively through the cell membrane[22,33]. Once re-uptaken by cells, both endocannabinoids are degraded through the action of the membrane-bound enzyme fatty acid amide hydrolase (FAAH)[33–37]. 2-Arachidonoylglycerol is also hydrolysed by other enzymes[33] or partly converted non-enzymatically into the 1(3)-isomer[32]. Arachidonic acid (AA), ethanolamine and glycerol, produced from anandamide and 2-AG hydrolysis, are rapidly re-esterified into membrane phospholipids, and so is the part of 2-AG that has diffused into the cell[22,33]. FAAH can be forced to catalyse the hydrolysis reverse reaction[35] and this could account for the synthesis of anandamide through the condensation of high concentrations of ethanolamine and AA, originally observed in FAAH-containing membrane preparations[36]. Abbreviations: AA, arachidonic acid; AC, adenylate cyclase; 2-AG, 2-arachidonoylglycerol; ArAPC,* sn*-1,2-di-arachidonoyl-PC; DAG, diacylglycerols; FAAH, fatty acid amide hydrolase; $InsP_2$, inositol-4,5-bisphosphate; $InsP_3$, inositol-1,4,5-trisphosphate; NaPE-PLD,* N*-acyl-phosphatidylethanolamine-selective phospholipase D; NArPE,* N*-arachidonoyl-PE; PA, phosphatidic acid ; PE, phosphatidylethanolamine; PI-PLC, PI-selective phospholipase C; PKA, protein kinase A; VIP, vasoactive intestinal peptide.*


Agonists (VIP)
Membrane-depolarizing stimuli
Ca2+
NMDA
Anandamide precursors
PE
Recycling
Gs
AC
ATP
cAMP
Ca2+
ArAPC
trans-acylase
AA + glycerol
PKA
?
NArPE
AA + ethanolamine
FAAH
Anandamide
NaPE-PLD
Anandamide and 2-AG metabolism
1-lyso-PC
2-AG
LysoPLC
PA
de novo synthesis (?) or phospholipid remodelling
2-AG precursors
Direct esterification into phospholipids
PA-hydrolase
sn-1-DAG Lipase
DAG
InsP3
PI-PLC
Ca2+
G
Agonists
PI
Carrier protein
Release and re-uptake
Anandamide
2-AG
Ca2+
Electrical activity, membrane-depolarizing stimuli

ethanolamine, which was initially substantiated by evidence gained from using cell-free systems[36]. However, this has never been demonstrated in living cells. Subsequent studies[27–30] have managed to characterize partially the Ca^{2+}-dependent *trans*-acylase that catalyses NArPE formation through the transfer of an arachidonate moiety from the *sn*-1 position of 1,2-*sn*-di-arachidonoyl-phosphatidylcholine to phosphatidyl-ethanolamine (Fig. 2). Stimulation of adenylate cyclase and cAMP-dependent protein kinase was shown to potentiate the Ca^{2+}-induced activation of the *trans*-acylase[28]. The discovery of this enzyme in brain areas containing high levels of anandamide[29], for example, the hippocampus, cortex, striatum and cerebellum[23], and the observation that its inhibition leads to suppression of NArPE formation by cortical neurones[29], support its role in the physiological biosynthesis of this endocannabinoid. It remains to be established, however, how the very low amounts of 1,2-*sn*-di-arachidonoyl-phosphatidylcholine present in the brain[27,29] can sustain the stimulus-induced biosynthesis of NArPE and anandamide. It has been suggested that Ca^{2+} influx into cells could lead to the synthesis of this phospholipid via activation of phospholipase A_2, release of AA and phospholipid remodelling[26].

2-Arachidonoylglycerol is also produced and released by mouse neuroblastoma and rat neuronal cells, but not by rat astrocytes, in a Ca^{2+}-dependent fashion upon ionomycin stimulation[31,32]. The biosynthesis of 2-AG is preceded by diacylglycerol formation and could follow both phospholipase C (PLC)-dependent[30,32] and -independent[31] pathways (Fig. 2). An *sn*-1-selective diacylglycerol lipase would be required in both cases[31,32]. Co-release of 2-AG and anandamide, which might result in increased flexibility of their possible neuromodulatory action, has been observed in cortical neurones following ionomycin treatment[22,32], but not in electrically stimulated hippocampal slices, where only the formation of 2-AG was reported[32]. In most cases the monoglyceride, which is 170-times more abundant than anandamide in rat whole brain[32], is synthesized in higher amounts than anandamide by stimulated neurones. However, it can be difficult to determine how much 2-AG is produced by cells in order to generate an extracellular cannabinoid signal, to terminate an intracellular diacylglycerol–protein kinase C-elicited signal, or to initiate an AA-mediated response, unless distinct metabolic routes are found for these three purposes. Further studies are, therefore, needed to clarify the biosynthesis of cannabimimetic 2-AG, whose action is terminated through diffusion into cells followed by both esterification into membrane phospholipids and enzymatic hydrolysis to AA and glycerol[33] (Fig. 2).

One enzyme plays a role in the degradation of both anandamide and 2-AG. Evidence[38] had suggested that the brain enzyme responsible for anandamide hydrolysis (originally named anandamide amidohydrolase[35]) could also react with the sleep-inducing factor, *cis*-9-octadecenoamide (oleamide)[34]. During later independent studies, the enzyme that catalysed oleamide hydrolysis was purified, cloned and sequenced. This protein was shown to catalyse the hydrolysis of anandamide more efficiently than the hydrolysis of oleamide[34], and the name fatty acid amide hydrolase (FAAH) was, therefore, suggested for this novel enzyme[34,38]. Subsequent studies demonstrated that, in rat tissues, the levels of FAAH mRNA – which are highest in brain and liver[34] – were correlated with: (1) the amounts of anandamide amidohydrolase[35], which further suggested that FAAH and anandamide amidohydrolase are the same, and (2) the presence of cannabinoid receptors in different brain regions, with mRNA for FAAH being predominantly found in the neocortex, amygdala, hippocampus, thalamus, and in some areas of the hypothalamus, brainstem and cerebellum[39]. Finally, recombinant FAAH was also found to catalyse efficiently the hydrolysis of 2-AG (Ref. 37), the levels of which can be elevated by specific FAAH inhibitors[33] in intact cells. The relative lack of selectivity of FAAH could have important physiological implications for its regulation by natural fatty acid amides and esters that do not bind to cannabinoid receptors but are recognized by the enzyme[33–35,37,38]. These natural fatty acid amides and esters are co-released or co-exist in nervous tissue with anandamide and 2-AG (Refs 18,22) and could increase the levels of these two endocannabinoids by competing for the same hydrolytic enzyme. This interaction could account for the weak cannabimimetic properties and for the potentiating effects on endocannabinoid actions that have recently been shown for oleamide[40].

Cannabinoid-receptor-mediated actions of endocannabinoids in neurones and astrocytes

In the nervous system, endocannabinoids exert most of their pharmacological actions by activating the CB_1 receptor, which is preferentially expressed in neurones of specific brain regions[7,21]. In transfected cells, the interaction with overexpressed CB_1 receptors and the subsequent activation of pertussis toxin-sensitive G-proteins allows anandamide to modulate negatively adenylate cyclase and voltage-sensitive Ca^{2+} channels and to activate the inwardly rectifying K^+ channel[12,13,41] (Fig. 3). Many of these intracellular actions have also been observed in rat brain tissues and cells constitutively expressing CB_1 receptors, particularly: (1) striatal slices, where anandamide inhibits forskolin-induced cAMP release[42], and (2) hippocampal neurones, where anandamide inhibits presynaptic N- and P/Q-type Ca^{2+} channels[43], and, by decreasing basal cAMP levels, induces tyrosine residue phosphorylation and focal adhesion kinase+ activation[46]. In striatal neurones, pertussis toxin treatment unmasks a G-protein-mediated linkage of CB_1 receptors with adenylate-cyclase stimulation[47]. Two other cannabinoid-receptor-mediated intracellular actions have also been reported for anandamide; these are stimulation of nitric oxide (NO) formation and AA release. The former effect was observed in leech and *Mytilus* ganglia and was blocked by SR141716A (Ref. 52). Induction of AA liberation can also be observed in cells that do not express cannabinoid receptors[12], and it was only recently, in mouse neuroblastoma N18TG2 cells[53], that experimental evidence was found for the actual participation of cannabinoid receptors in this anandamide and THC effect. 2-Arachidonoylglycerol also inhibits forskolin-induced cAMP formation in rat cortical neurones[32] and inward Ca^{2+} currents in differentiated neuroblastoma × glioma NG108-15 cells[44]. Interestingly, pulse stimulation of undifferentiated NG108-15 and N18TG2 cells with low nanomolar concentrations of the monoglyceride, but not anandamide, leads to a transient rise in intracellular Ca^{2+}

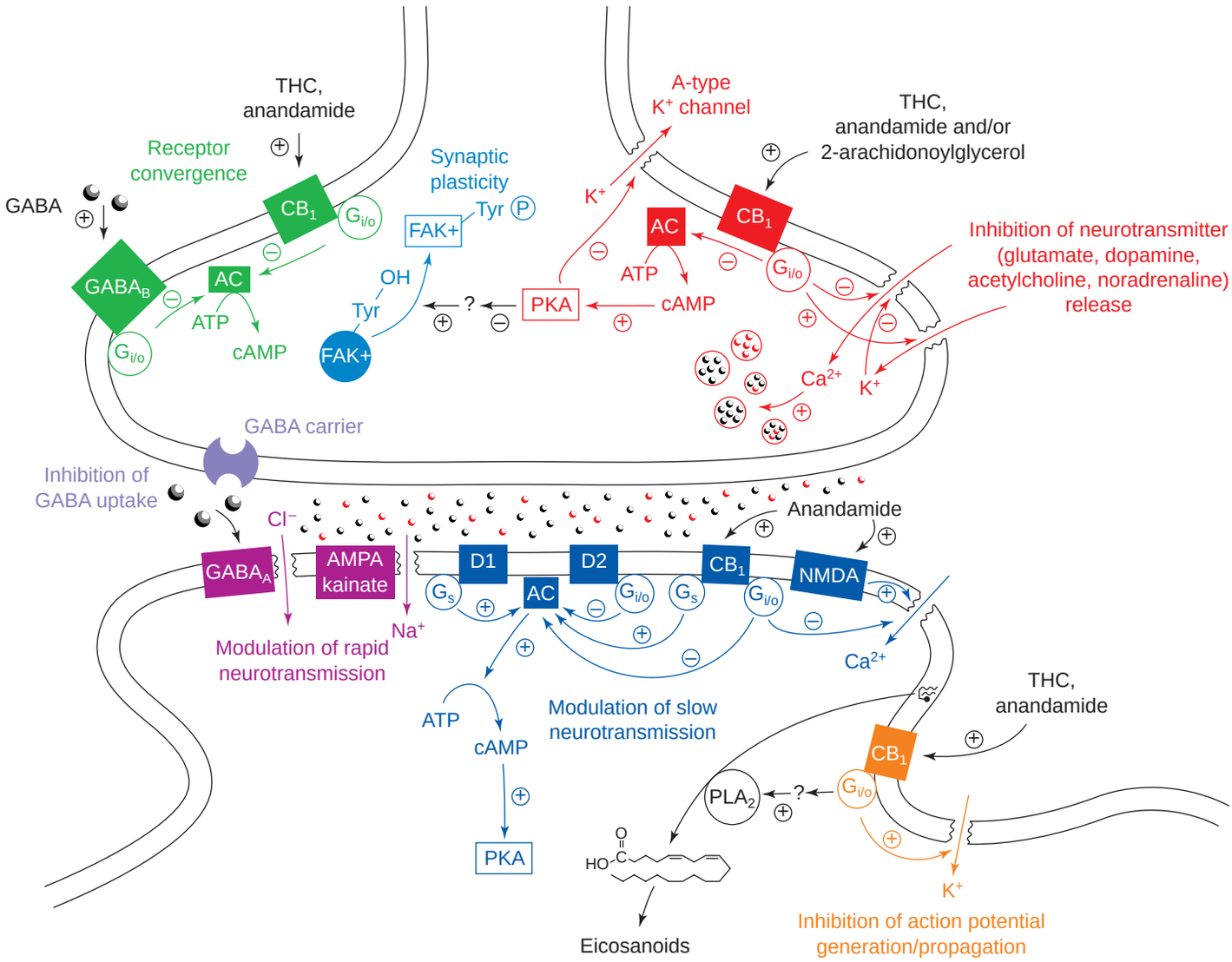


Fig. 3. ***Molecular bases of endocannabinoid neuromodulatory action.*** *At the presynaptic nerve terminal anandamide and 2-arachidonoylglycerol (2-AG), activate G-protein-coupled CB_1 receptors, modulating neuronal membrane permeability to Ca^{2+} and K^+ ions and the activity of adenylate cyclase (AC)[12,13,32,41–45], thereby affecting neurotransmitter release or action, or both, and both rapid and slow neurotransmission. Depending on the assay used, 1(3)-arachidonoyl glycerol is equipotent[32] or slightly less potent[45] than the 2-isomer. Once released by the depolarization of neurones, the two compounds, due to their lipophilicity, might behave, like other arachidonic acid (AA) derivatives, as autocrine or paracrine signals by acting on the same or neighbouring neurones or on astrocytes. In the hippocampus, inhibition of AC and subsequently cAMP-dependent protein kinase (PKA), can also lead to modulation of synaptic plasticity, for example, through increased tyrosine phosphorylation and subsequent activation of focal adhesion kinase+ (FAK+)[46]. G_s-mediated activation of AC was observed upon $G_{i/o}$ inhibition with pertussis toxin, and concurrent stimulation of D2 and CB_1 receptors in striatal slices leads to AC stimulation instead of inhibition[47]. In hippocampal slices, a direct potentiation of NMDA receptors by anandamide has also been observed[48]. By acting on CB_1 receptor-coupled K^+ channels, anandamide might also directly hyperpolarize smooth muscle cells[49]. Anandamide also affects intracellular AA and Ca^{2+} concentrations in astrocytes via pertussis toxin-sensitive mechanisms[50,51]. Abbreviations: AA, arachidonic acid; FAK+, focal adhesion kinase+; PLA_2, phospholipase A_2; THC, Δ^9(–)-tetrahydrocannabinol.*

levels via a CB_1-receptor-mediated, and possibly PLC-mediated, mechanism that has never been described previously for synthetic cannabinoids[45]. The possibility of this effect being due to interaction with a different CB_1-receptor-variant, such as that described by Shire *et al.*[54], has not been investigated.

Evidence for the presence of CB_1 receptors in astrocytes is still preliminary[7,21]. These cells might also contain a non-cannabinoid receptor accounting for anandamide inhibition of the propagation of a Ca^{2+} wave induced by glutamate. In fact, this effect, which was observed in striatal astrocytes[50], was not induced by synthetic cannabinoids and was blocked by pertussis toxin but not by the CB_1-receptor antagonist, SR141716A. This antagonist does counteract another pertussis toxin-sensitive effect of anandamide in cortical astrocytes, that is, stimulation of AA release[51], but only at concentrations five-times higher than anandamide. Thus, CB_1 receptors or yet-to-be discovered specific anandamide receptors could mediate the modulatory actions of anandamide on the homeostasis of astrocyte AA and Ca^{2+}, two important second messengers in neuronal–glial signalling.

Endocannabinoids as neuromodulators: where, when and why?

Some of the intracellular actions outlined above strongly suggest a role for endocannabinoids as modulators of neurotransmitter release and action (Fig. 3, Table 1). Their inhibitory effect on presynaptic voltage-sensitive Ca^{2+} channels might counteract the depolarization-induced release of neurotransmitters, whereas the facilitatory action on inwardly rectifying K^+ channels could reduce the likelihood of pre- and

TABLE 1. Possible neuromodulatory actions of endocannabinoids in the nervous system

Brain or peripheral region	Neuro-transmitter	Modulatory action	Possible CB_1 containing cell target	Endo-cannabinoids detected	Metabolic enzymes identified	Possible ultimate effect
Hippocampus	Glutamate	Inhibition of release	Glutamatergic CA3 and CA1 neurones	Anandamide, 2-arachidonoyl-glycerol	FAAH, *trans*-acylase, PI-PLC	Inhibition of LTP
	Acetylcholine	Inhibition of release	Cholinergic neurones of the septohippocampal perforant path in the dentate gyrus	As above	As above	Inhibition of learning and memory
Cerebellum	Glutamate[a]	Inhibition of NMDA receptor-mediated activation of P/Q type Ca^{2+} channel	Glutamatergic granule cells	Anandamide	Anandamide carrier (in granule cells) FAAH, *trans*-acylase	Inhibition of motor coordination, neuroprotection
Cortex	Glutamate[a]	As above	Cortical molecular layers	Anandamide, 2-arachidonoyl-glycerol (in cultured cortical neurones)	FAAH, *trans*-acylase anandamide carrier, PI-PLC, *sn*-1-DAG-lipase, MAG lipase	Inhibition of memory and motor behaviour, neuroprotection
Spinal cord	Glutamate[a]	Inhibition of release/NMDA receptor-mediated action	Neurones of the dorsal horn	2-arachidonoyl-glycerol (in dorsal root ganglia)	PI-PLC, *sn*-1-DAG-lipase, MAG lipase (dorsal root ganglia)	Spinal antinociception
Basal ganglia and substantia nigra	GABA[a]	Inhibition of re-uptake	GABAergic striatonigral and striatopallidal neurones	Anandamide (in the striatum)	FAAH (in the substantia nigra and globus pallidus) *trans*-acylase (in the striatum) anandamide carrier (in cultured striatal neurones)	Inhibition of locomotor activity, cataleptogenic activity
	Dopamine[b]	Inhibition of synthesis/release/ action	Dopaminergic nigrostriatal neurones	As above	As above	Inhibition of locomotor activity
	Dopamine[b]	Potentiation	As above	As above	As above	Induction of contralateral turning
Hypothalamus	Dopamine	Potentiation	Dopaminergic neurones of the tubero-infundibular system	No evidence	FAAH	Inhibition of prolactin release from the pituitary
Parasympathetic prejunctional fibres	Acetylcholine	Inhibition of release	Bladder, myenteric neurones	No evidence	No evidence	Inhibition of smooth muscle contractions
Sympathetic nervous system	Noradrenaline[c]	Inhibition of release (via NO release from renal endothelial cells)	Renal endothelial cells	Anandamide (in renal endothelial and mesangial cells)	FAAH (in renal endothelial and mesangial cells)	Hypotension
	Noradrenaline[c]	Inhibition of release from postganglionic fibres	Superior cervical ganglion	Anandamide (from macrophages)	No evidence	Hypotension, bradycardia
Invertebrate nervous system	Dopamine	Inhibition of release (via NO release)	*Mytilus* pedal ganglia, leech ventral ganglia	No evidence	No evidence	Unknown

[a,b,c]Other neuromodulatory actions have been suggested for Δ^9(–)-tetrahydrocannabinol (THC) (see Ref. 55 for a review), but there is no evidence as yet that they also occur with the endocannabinoids. These are: [a]inhibition of glutamate release from the subthalamic nucleus, from the cerebellar granule cells that stimulate Purkinje cells, and from the periaqueductal grey; [b]stimulation of dopamine release in the ventral tegmental area and from the nucleus accumbens, which possibly explain the rewarding effects of marijuana (Ref. 1) and [c]facilitation of α_2-adrenoceptor activation, inhibition of noradrenaline release or action, or both, in the hypothalamic medial preoptic area possibly leading to hypothermia and potentiation of noradrenaline action in the lateral hypothalamus possibly leading to appetite stimulation. Abbreviations: DAG, diacylglycerol; FAAH, fatty acid amide hydrolase; MAG, monoacylglycerol; NO, nitric oxide; PI-PLC, phosphoinositide-selective phospholipase C.

postsynaptic depolarization and of action potential generation[15,21]. Presynaptic inhibition of adenylate cyclase could also lead to activation of A-type K^+ channels, as shown for THC in cultured hippocampal cells[15], thus further inhibiting transmitter release. Anandamide can, therefore, inhibit AMPA/kainate-receptor-mediated

neurotransmission in hippocampal neurones by acting at the presynaptic level[56]. SR141716A enhances, and the synthetic cannabinoid, WIN55212-2, blocks, acetylcholine (but not GABA) release from hippocampal slices, which suggests that endogenous cannabinoids tonically inhibit the release of acetylcholine in the hippocampus[57]. These data could provide a molecular explanation for the CB_1-receptor-mediated impairment of working memory in rodents by anandamide[58], as well as for the improvement of short-term olfactory memory observed with SR141716A (Ref. 59). Interference with glutamate release could also contribute to the inhibitory action of anandamide on hippocampal LTP and transformation[60,61], and on memory consolidation[62]. However, the levels of anandamide in the hippocampus were not enhanced by electrical stimulation of presynaptic Schaffer collaterals, which instead led to the formation of 2-AG, also capable of inhibiting LTP (Ref. 32). Inhibition of glutamate-mediated neurotransmission at the level of dorsal root ganglia (which, as discussed in Refs 31,45, synthesize monoarachidonoylglycerols), or the periaqueductal grey, might also explain the analgesic properties of anandamide and 2-AG (Refs 9,17). In fact, the antinociceptive action of anandamide, unlike that of THC, does not seem to be mediated by endorphins[63], and the potent hyperalgesic properties of SR141716A and of CB_1-receptor-mRNA antisense oligonucleotides, which strongly support the existence of a tonic inhibition of thermal nociception by spinal endocannabinoids, are reversed by NMDA-receptor antagonists[64]. Finally, anandamide inhibition of NMDA-receptor-activated P/Q-type Ca^{2+} channels in cortical and cerebellar slices[48] could provide a mechanism for endocannabinoid impairment of motor coordination.

Modulation of dopamine- and GABA-mediated neurotransmission in the basal ganglia might explain the inhibition of motor activity[9,17] and the complex actions on turning behaviour observed for endocannabinoids in rodents (recently reviewed in Ref. 65). While the enhancement of GABA-mediated effects in the globus pallidus and the subsequent cataleptogenic activity might be due to inhibition of GABA re-uptake from striatopallidal terminals, several mechanisms have been proposed for the (sometimes) opposing actions of (endo)cannabinoids on extrapyramidal dopamine-mediated neurotransmission[65]. These include: (1) direct inhibition of striatal dopamine release[42]; (2) inhibition of dopamine D2- or D1-receptor-mediated action through reversal of adenylate cyclase inhibition[47] or activation, respectively; (3) indirect enhancement of striatal dopamine-mediated neurotransmission, suggested to intervene in anandamide induction of contralateral turning behaviour in mice; (4) inhibition of nigrostriatal tyrosine hydroxylase and decrease of the D1/D2 receptor ratio and (5) indirect inhibition of A9 dopaminergic neurones in the substantia nigra via potentiation of striato–nigral GABAergic afferent fibres.

Interference with dopamine-mediated neurotransmission might also lead to inhibition of memory consolidation by anandamide[62], whereas stimulation of tuberoinfundibular dopaminergic neurones in the arcuate nucleus[66], activation of the paraventricular nucleus[67], and inhibition of noradrenergic neurones in the medial preoptic area[66] could underlie the effects of anandamide on the hypothalamic–pituitary axis. These effects consist mainly of the enhancement of corticotropin releasing factor and adrenocorticotrophic hormone release[68] and the inhibition of production of prolactin, luteinizing hormone and growth hormone from the pituitary[66,67].

The smooth-muscle relaxant, hypotensive and bradycardic actions of anandamide have been associated with the downregulation of autonomic nervous functions (reviewed in Ref. 21). Activation of prejunctional or presynaptic CB_1 receptors by anandamide inhibits: (1) acetylcholine release from parasympathetic fibres that innervate the mouse urinary bladder and the guinea-pig myenteric plexus and (2) noradrenaline release from postganglionic sympathetic ganglia that innervate the heart and vasculature. A tonic control by endocannabinoids was suggested in the case of the former owing to the observation that SR141716A could produce slight stimulatory effects. Anandamide might also act through CB_1-receptor-mediated activation of endothelial NO formation and NO-mediated inhibition of neurotransmitter release, as shown for the inhibition of noradrenaline release from renal postganglionic sympathetic fibres[69]. Together with NO, prostanoids derived from AA produced from anandamide hydrolysis might also mediate the hypotensive action of this endocannabinoid, particularly in cerebral arterioles[70]. While the occurrence of endocannabinoids in autonomic fibres has not yet been investigated, other possible sources of vasorelaxant anandamide have been proposed. These are: (1) macrophages (where the endocannabinoid is produced *in vitro*, by ionomycin stimulation[71], and *ex vivo*, following haemorrhagic shock[72]) and (2) endothelial cells, which release anandamide[69] and also a factor whose direct hyperpolarizing action on smooth muscle cells can be blocked by SR141716A (Ref. 49). The possibility that the long-sought-after endothelium-derived hyperpolarizing factor might be anandamide or another endocannabinoid (for example, 2-AG, which is also released by endothelial cells[73]) is still in dispute[49].

What is behind the endogenous cannabinoid system?

Six years of intensive research on endocannabinoids have not yet succeeded in identifying the key physiological function of the endogenous cannabinoid system. Reports of the actions of anandamide and 2-AG in non-nervous tissues, namely in the reproductive and immune systems, have not been discussed in this article and widen the range of the possible roles played by these metabolites (for a review see Ref. 74). Three of the typical behavioural effects of marijuana smoking, appetite stimulation, relief of anxiety, and sedation, have been correlated previously with the presence of cannabinoid receptors in the hypothalamus and the limbic system. More recently, these effects have been linked with a possible tonic endocannabinoid stimulation of sucrose and ethanol intake[75], inhibition of anxiety-like responses[76] and decrease of arousal[77] in rodents. Moreover, anandamide has been shown to inhibit aggressiveness in singly housed mice[78]. Therefore, on the basis of the findings reviewed here, it might be intriguing to speculate that the endocannabinoids have a general function as stress-recovery factors, which they exert mostly through the relief of some typical stress-induced responses at the level of both central and peripheral

nervous systems. Also, a neuroprotective role for anandamide or *N*-acylethanolamines, or both, has been suggested by the following findings: (1) anandamide inhibition of NMDA-receptor-mediated Ca^{2+} influx[48]; (2) *N*-palmitoylethanolamine protection against glutamate-induced excitotoxicity[79] and (3) increased levels of these compounds following glutamate stimulation or cell injury[26,28,80]. Thus, 'relax, eat, sleep, forget and protect' might be some of the messages that are produced by the actions of endocannabinoids, alone or in combination with other mediators. However, several aspects of endocannabinoid biosynthesis, action and co-localization with other neurotransmitters still need to be investigated, and it will probably take many years of coordinated research among biochemists, neurophysiologists and psychiatrists to evaluate critically this or other hypotheses that relate to the physiological significance of the endogenous cannabinoid system.

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