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The molecular logic of endocannabinoid signalling

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The Cannabis plant has been used in Europe since antiquity, mostly to make cordage and fabric, but first attracted the attention of European scientists when Napoleon’s troops brought back from Egypt intriguing accounts of its psychotropic activity. In 1810, a member of Napoleon’s Commission des Sciences et des Arts wrote1: “For the Egyptians, hemp is the plant par excellence, not for the uses they make of it in Europe and many other countries, but for its peculiar effects. The hemp cultivated in Egypt is indeed intoxicating and narcotic.”

Before long, detailed descriptions of the plant’s properties began to appear2,3 and Cannabis extracts were introduced to the medical community. An 1848 commentary of the British Pharmacopoeia outlined quite accurately the psychotropic effects of Cannabis and pointed out its merit as an analgesic and antispasmodic4:

“Numerous observers have described the Indian hemp as producing in the natives of the East, who familiarly use it instead of intoxicating spirits, sometimes a heavy, lazy state of agreeable reverie, from which the individual may be easily roused to discharge any simple duty — sometimes a cheerful, active state of inebriation causing him to dance, sing and laugh, provoking the veneral appetite, and increasing the desire for food — and sometimes a quarrelsome drunkenness, leading to acts of violence. During this condition pain is assuaged and spasm arrested. […] On the whole, it is a remedy which deserves a more extensive inquiry than any hitherto instituted.”

The inquiry into the active chemical constituents of Cannabis turned out to be more time consuming than expected. Many other plant-derived compounds, such as morphine and atropine, had long been identified when the Cannabis plant finally yielded its active principle, the terpenoid derivative ∆9-tetrahydrocannabinol (THC)5,6 (FIG. 1).

The psychoactive properties of THC were recognized immediately, but the drug’s unique chemical structure offered no hints as to its mechanism of action. To complicate matters further, the hydrophobic nature of THC delayed experimentation and indicated that the compound might act by influencing membrane fluidity, rather than by combining with a specific receptor. This impasse was resolved by the development of new classes of potent and selective THC analogue7 (FIG. 1), which led eventually to the pharmacological identification of cannabinoid-sensitive sites in the brain8.

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The CB1 cannabinoid receptor was molecularly cloned from rat brain in 1990 (REF. 9) and its immune-system counterpart, the CB2 receptor, was identified by sequence homology three years later10. These discoveries not only established the mechanism of action of THC, thereby fuelling the development of subtype-selective agonists and antagonists (FIG. 1), but they also initiated a hunt for brain-derived cannabinoid ligands. Surprisingly, the first THC-like factor to be isolated was a lipid, rather than the peptide that had been expected on the basis of the precedent set by morphine and the enkephalins. It was identified as the amide of arachidonic acid.
An important class of membrane (20 carbons, 4 double bonds). Polyunsaturated fatty acids (without double bonds) are palmitic (16 carbons) and stearic (18 carbons). Examples of unsaturated fatty acids include oleic (18 carbons, one double bond) and arachidonic (20 carbons, 4 double bonds).

**Phosphatidylyethanolamine (PE)**

An important class of membrane phospholipids comprising a glycerol skeleton linked to two fatty acid residues, phosphoric acid and ethanolamine.

with ethanolamine, and named anandamide after the Sanskrit word for bliss, ananda11 (FIG. 2).

This small lipid molecule resembled no known neurotransmitter, but it did share structural features with the **Eicosanoids**, mediators of inflammation and pain with various functions in neural communication12. Though initially controversial13, the signalling roles of anandamide were confirmed by the elucidation of the compound’s unique metabolic pathways and the demonstration of its release in the live brain14–16. As the search for THC-like compounds continued, other bioactive lipids were extracted from animal tissues. These include 2-arachidonoylglycerol (2-AG)17,18, noladin ether19, virodhamine20 and N-arachidonoyldopamine21 (FIG. 2).

In this article, I review the synthesis, release and deactivation of the endogenous cannabinoids (also called endocannabinoids). I then outline the properties and distribution of brain CB receptors. Last, I describe the function of the endocannabinoids as local modulators of synaptic activity and their contribution to memory, anxiety, movement and pain.

**Synthesis**

Anandamide. The membranes of plant cells contain a family of unusual lipids that consist of a long-chain **Fatty Acid** tethered to the head group of **Phosphatidylyethanolamine (PE)** through an amide bond. When attacked by a **Phospholipase D (PLD)** enzyme, these membrane constituents generate a set of **Fatty Acid Ethanolamines**, which are used by plants as intercellular signalling molecules. They are released from cells in response to stress or infection, and stimulate the expression of genes engaged in systemic plant immunity22. This ancestral biochemical device is conserved in mammalian cells, which use the ethanolamine of arachidonic acid, anandamide, as a primary component of the endocannabinoid signalling system.

Anandamide formation in neurons is a two-step process, which parallels fatty acid ethanolamine production in plants23,24 (FIG. 3). The first step is the stimulus-dependent cleavage of the phospholipid precursor **N-arachidonyl-PE**. This reaction is mediated by an uncharacterized PLD and produces anandamide and phosphatidic acid, a metabolic intermediate that is used by cells in the synthesis of other glycerol-derived phospholipids. Genes encoding two PLD isoforms have been cloned in mammals23, but it is not known whether either of these enzymes is responsible for anandamide synthesis.

The brain contains tiny quantities of **N-arachidonyl-PE** (20–40 pmol g−1)23,24 — probably too little to sustain anandamide release for an extended time. The cellular stores of this precursor are replenished by the enzyme **N-acylethanolamine phosphatase**, which catalyses the intermolecular passage of an arachidonic acid group from the sn-1 position of **Phosphatidylcholine** to the head group of PE24,22,24 (FIG. 3). In cultures of rat cortical neurons, two intracellular second messengers control **NAT** activity: Ca2+ and cyclic AMP. Ca2+ is required to engage **NAT**, which is inactive in its absence, whereas cAMP works through protein kinase A-dependent phosphorylation to enhance **NAT** activity24. Although catalysed by separate enzymes, the syntheses of anandamide and its parent lipid are thought to proceed in parallel because Ca2+-stimulated anandamide production is generally accompanied by **de novo** formation of **N-arachidonyl-PE**23,24.

As expected of a Ca2+-activated process, anandamide formation can be elicited by Ca2+ ionophores, which carry Ca2+ ions across cell membranes. For example, in cultures of rat striatal neurons labelled by incubation with [3H]ethanolamine, the Ca2+ ionophore ionomycin stimulates accumulation of [3H]anandamide14. A similar stimulation is produced by kainate (a glutamate receptor agonist), 4-aminopyridine (a K+ channel blocker) or membrane depolarizing concentrations of K+, and can be prevented by chelating extracellular Ca2+ [REFS 14,26]. The Ca2+ dependence of anandamide synthesis was also demonstrated using **Microdialysis**. Administration of a high-K+ pulse in the rat striatum caused a reversible increase in interstitial anandamide concentrations, which was prevented by removal of Ca2+ from the perfusing solution15.

Although neural activity induces anandamide release in a Ca2+-dependent manner, Ca2+ entry into neurons is not the only determinant of anandamide generation: there is evidence that G-protein-coupled receptors can...
also trigger this process. For example, application of the dopamine D₂-receptor agonist quinpirole causes an eightfold increase in anandamide outflow in the rat striatum, which is prevented by the D₂-receptor antagonist raclopride. This response is accompanied by an elevation in tissue anandamide content, indicating that it might be due to anet increase in anandamide formation rather than to extracellular release of preformed anandamide. Muscarinic acetylcholine receptors and metabotropic glutamate receptors can also cause endocannabinoid release in hippocampal slices in a Ca²⁺-independent manner, but the substance(s) involved have not been identified.

How does occupation of D₂ receptors initiate anandamide synthesis? Inhibition of cAMP formation, a hallmark of D₂-receptor signalling, is unlikely to be responsible for this effect because cAMP positively regulates NAT activity. Alternatively, D₂ receptors could interact with the Rho family of small G proteins to stimulate PLD activity, or they might engage βγ-subunits of G proteins to activate phospholipase C₁₂₆ (PLC₁₂₆). PLC₁₂₆ catalyses the cleavage of phosphatidylinositol-4,5-bisphosphate to produce inositol-1,4,5-trisphosphate, which might then recruit the NAT/PLD pathway by mobilizing Ca²⁺ from internal stores.

2-Arachidonoylglycerol. Like other monooacylglycerols, 2-AG is at the crossroads of multiple routes of lipid metabolism, where it can serve interchangeably as an end-product for one pathway and precursor for another. These diverse metabolic roles can explain its high concentration in brain tissue (about 200-fold greater than anandamide)²¹,²², and imply that a significant fraction of brain 2-AG is engaged in housekeeping functions rather than in signalling.

The place occupied by 2-AG at central intersections of lipid metabolism also complicates efforts to define the biochemical pathway(s) responsible for its physiological synthesis. There is, however, enough information to indicate possible routes (FIG. 4). The first begins with the phospholipase-mediated formation of 1,2-diacylglycerol (DAG). This product regulates protein kinase C activity—an important second messenger function—and is a substrate for two enzymes: DAG kinase, which attenuates DAG signalling by catalysing its phosphorylation to phosphatidic acid; and DAG lipase (DGL), which hydrolyses DAG to monoacylglycerol. The fact that drug inhibitors of PLC and DGL block Ca²⁺-dependent 2-AG accumulation in rat cortical neurons indicates primary involvement of this pathway in 2-AG formation.

An alternative pathway of 2-AG synthesis begins with the production, mediated by phospholipase A₁ (PLA₁)³⁵,³⁶, of a 2-arachidonoyl-LYSOPHOSPHOLIPID, which might be hydrolysed to 2-AG by lyso-PLC activity (FIG. 4). Although there is no direct evidence for this mechanism in 2-AG formation, the high level of PLA₁ expression in brain tissue makes it an intriguing target for future investigation. In addition to the phospholipase-operated pathways outlined above, monooacylglycerols can be produced by hormone-sensitive lipase acting on triacylglycerols or by lipid phosphatases acting on lysophosphatidic acid. In general, however, these enzymes preferentially target lipids that are enriched in saturated or monounsaturated fatty acids, rather than the polyunsaturated species that would give rise to 2-AG.

Irrespective of its exact mechanism, neuronal 2-AG production can be initiated by an increase in the concentration of intracellular Ca²⁺. In cultures of rat cortical neurons, the Ca²⁺-ionophore ionomycin and the glutamate receptor agonist N M DA (N-methyl-D-aspartate) stimulate 2-AG synthesis in a Ca²⁺-dependent manner. Likewise, in freshly dissected hippocampal slices, high-frequency stimulation of the SCHÄFER COLLATERALS produces a Ca²⁺-dependent increase in tissue 2-AG content. Importantly, this treatment has no effect on the concentrations of non-cannabinoid monooacylglycerols, such as 1(3) palmitoyleglycerol, which indicates...
REVIEWS

Figure 4 | Pathways of 2-arachidonoylglycerol (2-AG) formation in neurons. One possible sequence of reactions, shown on the left, includes the cleavage of phosphatidylinositol (PI) to yield 1,2-diacylglycerol (DAG), catalysed by a phospholipase such as phospholipase C (PLC), and the subsequent conversion of DAG to 2-AG, catalysed by diacylglycerol lipase (DGL). An alternative route, shown on the right, comprises the formation of a 2-arachidonoyl-lysophospholipid such as lyso-PI, catalysed by phospholipase A1 (PLA1), followed by the hydrolysis of the lysophospholipid to 2-AG, catalysed by lyso-PLC.

MONOACYLGLYCEROLO

A glycerol derivative in which one of the hydroxyl groups is linked to a fatty acid residue by an ester bond.

LYSOPHOSPHOLIPID

A phospholipid containing only one fatty acid chain. Examples include lysophosphatidic acid and lysophosphatidyl-ethanolamine.

SCHAEFFER COLLATERALS

Axons of the CA3 pyramidal cells of the hippocampus that form synapses with the apical dendrites of CA1 neurons.

FACILITATED DIFFUSION

A common mechanism of transmembrane transfer that involves a protein carrier, but does not require expenditure of cellular energy.

VANILLOID RECEPTORS

Membrane receptor channels permeable to monovalent cations. They are activated by noxious heat and capsaicin, the active constituent of hot chili peppers.

that 2-AG formation is not due to a generalized increase in the rate of lipid turnover. Furthermore, high-frequency stimulation does not alter hippocampal anandamide concentrations, indicating that the synthesis of 2-AG and anandamide can be independently regulated. In further support of this idea, activation of D2 receptors — a potent stimulus for anandamide formation in the rat striatum — has no effect on striatal 2-AG concentrations.

Other putative endogenous ligands. Noladin ether is an ether-linked analogue of 2-AG that binds to and activates CB1 receptors (Fig. 2). Its pathway of formation has not been characterized, and its occurrence in the normal brain has been questioned. Virodhamine, the ester of arachidonic acid and ethanolamine (Fig. 2), might act as an endogenous CB2 antagonist. Its presence in brain tissue has been documented, but is intriguing because this chemically unstable molecule is rapidly converted to anandamide in aqueous environments. The mechanism of its synthesis is unknown, and its deactivation might share anandamide's pathways of uptake and intracellular hydrolysis. Finally, the endogenous vanilloid agonist, N-arachidonoyl-dopamine, also exhibits affinity for cannabinoid receptors in vitro (Fig. 2).

Release from neurons

How are endocannabinoids released from cells and how do they reach their targets? Classical transmitters and neuropeptides can diffuse through the water-filled space that surrounds neurons, but hydrophobic compounds such as anandamide and 2-AG tend to remain associated with lipid membranes. One possibility is that endocannabinoids might not leave the cell where they are produced; rather, they could move sideways within the plasma membrane until they collide with membrane-embedded CB1 receptors. This hypothesis is supported by the role of an intramembranous amino-acid residue (lysine-192) in the binding of anandamide to CB1 (REF. 38), as well as by the finding that certain cannabinoid agonists can approach the receptor by lateral membrane diffusion. Nevertheless, it does not account for two pieces of evidence. First, anandamide is found in incubation media of cells and in brain interstitial fluid, implying that it can overcome its tendency to partition in membranes. Perhaps more importantly, physiological experiments have shown that an endocannabinoid substance does leave postsynaptic cells to activate CB1 receptors on adjacent axon terminals. This unidentified compound might travel as far as 20 μm from its cell of origin before being eliminated.

If endocannabinoids are released from neurons, what is the mechanism of their release? The fact that plasma membranes contain precursor molecules for both anandamide and 2-AG indicates that they could leave the cell as soon as they are formed. Extracellular lipid-binding proteins such as the lipocalins, which are expressed at high levels in the brain, might facilitate this step and help to deliver endocannabinoids to their cellular targets. Although this scenario awaits confirmation, it does mirror what happens in the bloodstream, where anandamide's movements are made possible by its reversible binding to serum albumin.

Deactivation

Two mechanisms cooperate in attenuating endocannabinoid signalling in the brain: carrier-mediated transport into cells and intracellular hydrolysis (Fig. 5).

Transport. Anandamide and 2-AG can diffuse passively through lipid membranes, but this process is accelerated by a rapid and selective carrier system that is present in both neurons and glial cells. Although it is superficially similar to other transmitter systems, endocannabinoid transport is not driven by transmembrane Na+ gradients, indicating that it might be mediated by a facilitated diffusion mechanism. In this respect, neural cells seem to internalize anandamide and 2-AG in a manner similar to fatty acids, eicosanoids and other biologically relevant lipids, by using energy-independent carriers. Several lipid-carrier proteins have been molecularly cloned, inspiring optimism that, despite current controversy (BOX 1), endocannabinoid transporter(s) will eventually be characterized.

Meanwhile, to gain insight into the role of transport in endocannabinoid inactivation, we can rely on an expanding series of pharmacological transport inhibitors. The prototype is AM 404, which slows the elimination of both anandamide and 2-AG, magnifying their biological effects. This inhibitor has helped to unmask important roles of the endocannabinoid system in the regulation of neurotransmission and synaptic plasticity, but suffers from various limitations, including an affinity for vanilloid receptors and susceptibility to enzymatic attack by fatty acid amide hydrolase (FAAH). These limitations have prompted an ongoing search for more selective and stable analogues.
**Monoacylglycerol lipase.** The pig brain contains two chromatographically distinct 2-AG-hydrolysing activities, one of which is probably due to the enzyme monoacylglycerol lipase (MGL). The rat brain isofrom of this cytosolic serine hydrolase has been characterized both molecularly and morphologically.

FAAH is widely distributed in the rat brain, where it is expressed at high concentrations in cell bodies and dendrites of principal neurons. In the hippocampus, neocortex and cerebellum, FAAH-positive cell bodies are juxtaposed to axon terminals that contain CB1 receptors, indicating not only that FAAH participates in the inactivation of neurally generated anandamide, but also that this process occurs postsynaptically. This idea can now be tested in FAAH-deficient mice or using selective FAAH inhibitors with long-lasting systemic actions.

FAAH is an intracellular membrane-bound serine hydrolase that breaks down anandamide into arachidonic acid and ethanolamine (Fig. 5). It has been molecularly cloned and its catalytic mechanism, which allows it to recognize a broad spectrum of amide and ester substrates, has been elucidated in detail. Particularly notable is FAAH’s ability to hydrolyse bioactive fatty amides, which do not bind to any of the known cannabinoid receptors: these include the satiety factor oleoylethanolamide and the anti-inflammatory/analgesic mediator palmitoylethanolamide. FAAH tightly controls brain concentrations of these compounds, but the functional significance of this regulation is unknown.

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however, endocannabinoid-mediated suppression of GABA release in hippocampal slices seems primarily to involve N-type Ca\(^2+\) channels\(^{35}\).

Cannabinoid regulation of voltage-gated K\(^+\) currents\(^{35}\) is also implicated in presynaptic inhibition at GABA\(^{30}\) and glutamate synapses. The latter include parvocellular interneurons in the cerebellum, as well as synapses in the nucleus accumbens and lateral amygdala\(^{34,36}\) (FIG. 7b). The sensitivity of these responses to pertussis toxin implies that they are mediated by G\(_o\) proteins, but it is still unclear whether transduction is direct (β-γ subunit-mediated) or indirect (second messenger-mediated). Inhibition of cAMP formation does not seem to be involved\(^{35,36}\).

On the other hand, cAMP can contribute to the regulation of neuronal gene expression by CB\(_1\). This process, which is necessary to produce lasting changes in synaptic strength, depends on the recruitment of complex networks of intracellular protein kinases\(^{35}\). Two components of these networks, extracellular signal-regulated kinase (ERK) and focal adhesion kinase (FAK), become activated when hippocampal slices are treated with cannabinoid agonists\(^{88,89}\). This activation is mimicked by activators of MAPK (ERK) and focal adhesion kinase (FAK) in synaptic plasticity indicates that these protein kinases could participate in the changes in gene expression and the persistent neural adaptations that accompany cannabinoid administration\(^{90}\).

CB\(_1\) distribution
In the rodent and human cortices, CB\(_1\) receptors are primarily found on axon terminals of cholecystokinin-8 (CCK-8)-positive GABA interneurons\(^{90-95}\). This expression pattern dominates the neocortex, hippocampal formation and amygdala, where nerve terminals that form excitatory synapses are ostensibly devoid of CB\(_1\). Immunoreactivity\(^{93,94}\). However, there is evidence that excitatory terminals in these regions do contain the receptor; for example, cannabinoid agonists reduce glutamatergic transmission in the amygdala of normal mice, but fail to do so in CB\(_1\)-deficient mutants\(^{96}\). In addition, low concentrations of CB\(_1\) messenger RNA have been found in many neurons of the cortex that do not contain GABA\(^{32}\).

CB\(_2\) receptors are also expressed at very high levels throughout the basal ganglia. In the striatum they are localized to three distinct neuronal elements: glutamatergic terminals originating in the cortex\(^{70,72}\), local-circuit GABA interneurons (‘fast-spiking’ interneurons that do not express CCK-8)\(^{96}\) and axon terminals of GABA projection neurons (‘medium spiny neurons’)\(^{70}\). Medium spiny neurons project striatal outflow nuclei, where CB\(_2\) receptors are especially abundant; for example, in the globus pallidus they outnumber dopamine D\(_2\) receptors by a factor of 45 (REF. 97).

In the cerebellum, CB\(_1\) is present on excitatory terminals of climbing and parallel fibres (but not on their postsynaptic partners, the Purkinje neurons) as well as on GABA interneurons\(^{70,71}\). Smaller numbers of CB\(_2\) receptors are also found in the thalamus (especially in the anterior dorsal nucleus and habenula), hypothalamus (ventromedial and anterior nucleus), midbrain (periaqueductal grey and superior colliculus), medulla (dorsal vagal complex and rostral ventromedial medulla) and spinal cord (dorsal horn)\(^{70,73}\). Last, CB\(_1\) is expressed in peripheral sensory neurons\(^{98}\), where it is localized in cells that express N\(_2\), a protein marker of mechanosensitive A\(_\beta\) fibres\(^{99}\).

Another brain receptor?
A few cannabinoid effects persist in CB\(_1\)-null mice, implying that this receptor might not act alone in mediating brain cannabinoid signalling\(^{32,100}\). Although cannabinoid agonists lose their ability to inhibit GABA and glutamate transmission in some brain regions of adult CB\(_1\)-knockout mice\(^{38}\), they can still reduce excitatory transmission in the hippocampal CA1 field of these animals\(^{28,102}\). This discrepancy is reinforced by the finding that GABA and glutamate synapses in CA1 respond in different ways to cannabinoid drugs. For example, cannabinoid depression of excitatory currents is blocked by CB\(_1\) antagonists, whereas depression of inhibitory currents is not\(^{32}\). These results make a persuasive case for the existence of a dopaminergic cannabinoid-sensitive site that is distinct from CB\(_1\) (sometimes called ‘CB\(_2\)’), but other evidence appears to contradict them; for example, in newborn CB\(_1\)-null mice, cannabinoid agonists affect neither GABA nor glutamate transmission\(^{104}\). Although this difference could be due to the developmental stage of the preparation used -- adult\(^{102}\) versus one-week-old mice\(^{104}\) -- more studies are needed to establish whether the CB\(_1\) site is molecularly distinct from CB\(_2\). A novel cannabinoid site has also been identified in the vascular endothelium\(^{105}\), but seems to be different from CB\(_3\) because it is not antagonized by capsaicin\(^{106}\) or activated by the CB\(_3\)/CB\(_3\) agonist WIN-55212-2 (REF. 106).

A local message
Outside the brain, the endocannabinoids are produced on demand and act on cells located near their site of synthesis. For example, they are formed by circulating
leukocytes and platelets, and induce vascular relaxation by interacting with cannabinoid receptors on the surface of neighbouring endothelial and smooth muscle cells.

Similar paracrine actions are thought to occur in the CNS, where the endocannabinoids might mediate a localized signalling mechanism through which principal neurons modify the strength of incoming synaptic inputs.

Regulation of GABA transmission

Hippocampus modulation of memory. When a pyramidal neuron in the CA1 field of the hippocampus is depolarized, the inhibitory GABA inputs received by that cell are transiently suppressed. This phenomenon, called depolarization-induced suppression of inhibition (DSI), is initiated postsynaptically by voltage-dependent influx of Ca\(^{2+}\) into the soma and dendrites of the neuron, but is expressed presynaptically through inhibition of transmitter release from axon terminals of GABA interneurons. This indicates that a chemical messenger generated during depolarization of the pyramidal cell must travel backwards across the synapse to induce DSI (FIG. 8).

There is evidence that this retrograde signalling process involves an endocannabinoid substance, possibly 2-AG. First, CB\(_1\) agonists mimic DSI, whereas CB\(_1\) antagonists block it. Second, DSI is absent in CB\(_1\)-deficient mice. Third, the GABA interneurons that are implicated in DSI express high levels of CB\(_1\) receptors, which are localized to their axon terminals. Fourth, neural activity and Ca\(^{2+}\) entry stimulate the hippocampal synthesis of 2-AG, but have no effect on anandamide concentrations. Nevertheless, we still don't know whether the endocannabinoid actually crosses back to the presynaptic nerve ending or is produced there by the action of another, unidentified retrograde signal.

The fact that DSI is induced in vitro by levels of neural activity that could also be encountered in vivo indicates that this process might have a role in normal brain function. Although this idea is still questioned, various results link DSI to the regulation of hippocampal gamma oscillations. These network oscillations are coordinated by CB\(_1\)-positive GABA interneurons and are influenced by cannabinoid agonists, raising the possibility that an endocannabinoid substance might modulate their expression and be involved in the organization of hippocampal cell assemblies. Another function of DSI might relate to synaptic plasticity. By weakening GABA-mediated inhibition, DSI could facilitate the induction of long-term potentiation in individual CA1 pyramidal neurons; this might contribute in turn to the formation of place fields or to other forms of hippocampus-dependent learning. Such a cognitive-enhancing action would not contradict the well-known amnesic effects of cannabinoid drugs as the latter might result from a generalized, circuit-independent activation of CB\(_1\) receptors in the hippocampus and other brain areas.

Outside the hippocampus, endocannabinoid-mediated DSI has been shown to occur at interneuron–principal cell synapses of the cerebellum and probably will soon be discovered elsewhere.

Amygdala: modulation of emotions. CB\(_1\)-bearing interneurons are selectively localized to a subdivision of the amygdala called the basolateral complex, a key station in the neural circuitry that processes emotions and a primary site of cannabinoid analgesia. This localization, and the fact that CB\(_1\) inactivation causes anxiety-like and aggressive responses in rodents, indicate that the endocannabinoid system might influence affective states through changes in the amygdala's efferent activity. This idea is further supported by two findings: first, presentation of anxiogenic stimuli increases anandamide and 2-AG concentrations in the mouse amygdala; second, FAAH inhibitors exhibit marked anxiolytic-like properties in rats.

Locally formed endocannabinoids could modify the amygdala's output in two complementary ways.

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**Diagram**

- **Figure 7** Regulation of presynaptic ion channel activities by CB\(_1\) cannabinoid receptors.
  - **a** At synapses between GABA (\(\gamma\)-aminobutyric acid) interneurons and pyramidal cells in the CA1 field of the hippocampus, activation of CB\(_1\) receptors can initiate a series of intracellular events, which include (1) activation of G-protein βγ subunits, (2) closure of voltage-gated Ca\(^{2+}\) channels and (3) inhibition of GABA release.
  - **b** At parallel fibre–Purkinje cell synapses in the cerebellum, CB\(_1\) activation can (1) engage G-protein α-subunits that (2) cause the opening of K\(^{+}\) channels; the resulting membrane hyperpolarization can (3) reduce Ca\(^{2+}\) entry and inhibit glutamate release. Mechanisms similar to those illustrated above are thought to underlie cannabinoid-mediated inhibition of neurotransmitter release in other brain regions.
they could depress glutamate release from axon terminals originating in the cortex and other brain regions. In addition, by reducing GABA release from basolateral interneurons, they might disinhibit GABA cells in the adjacent intercalated nuclei and consequently decrease the activity of their postsynaptic targets, the pyramidal neurons in the central nucleus of the amygdala, which constitute the structure’s primary efferent pathway.

Basal ganglia: modulation of motor activity. The terminal fields of striatal projection neurons contain the highest densities of CB1 receptors in the brain. Here, local administration of cannabinoid agonists inhibits GABA release and profoundly affects motor behaviours. Membrane depolarization and dopamine D2-receptor activation stimulate striatal anandamide formation, indicating that this endocannabinoid might contribute to the regulation of basal ganglia function. In agreement with this hypothesis, the CB1 antagonist rimonabant enhances the stimulation of movement that is induced in rats by dopamine agonists, whereas the endocannabinoid transport inhibitor AM404 attenuates this stimulation in a CB1-dependent manner.

Anandamide might act at multiple sites in the basal ganglia, including GABA projection neurons, corticostriatal glutamatergic terminals and local-circuit interneurons. Local-circuit interneurons are particularly notable because of their functional resemblance to CB1-positive interneurons in the hippocampus, with which they share not only a GABA-containing phenotype, but also the ability to discharge high-frequency bursts of action potentials that can inhibit firing in large assemblies of projection cells. Does locally released anandamide gain access to these interneurons? Or does it primarily act on medium spiny cells and their cortical afferents? We don’t know yet. But these unanswered questions do not diminish the significance of striatal endocannabinoid signalling, which is further highlighted by the effectiveness of cannabinoid agonists in the symptomatic treatment of levodopa-induced dyskinesias and Tourette’s syndrome, two disorders with strong striatal underpinnings.

Hindbrain: central analgesia. Besides their actions in the amygdala, cannabinoid agonists can influence the central processing of pain by interacting with CB1 receptors in the periaqueductal grey, rostral ventromedial medulla and spinal trigeminal nucleus. At each of these sites, CB1 activation depresses GABA release through a presynaptic mechanism, without causing significant changes in somatic membrane conductances. In the trigeminal nucleus, glycnergic transmission also is inhibited. Painful stimuli elicit anandamide release in the rat periaqueductal grey, and systemic administration of CB1 antagonists produces hyperalgesia in rats and mice. So, noxious stimuli can engage a central analgesic circuit operated by the endocannabinoids, which, working in combination with a parallel mechanism in the periphery, could underlie the analgesic properties of cannabinoid drugs.

Regulation of glutamate transmission. Principal neurons in the hippocampus and cerebellum use endocannabinoids to carry out a signalling process that is analogous in mechanism, but opposite in sign, to DSI, called depolarization-induced suppression of excitation (DSE). Like DSI, DSE is induced by neuronal depolarization, it consists of a transient depression in neurotransmitter release, and it requires a retrograde endocannabinoid messenger. But unlike DSI, DSE targets glutamatergic rather than GABA axon terminals, and results therefore in reduced excitatory input to the affected cells. Do DSI and DSE occur simultaneously in a single neuron and, if so, how are they coordinated? In cerebellar Purkinje cells, the two opposing phenomena can be elicited by similar stimulation protocols and so are likely to coexist. Although they might be topographically segregated along the longitudinal axis of the neuron, the significance of their coexistence is not known. On the other hand, in the hippocampus, the induction of DSE requires longer periods of depolarization than does DSI, and its magnitude is smaller. This could be explained by the lower sensitivity of glutamatergic terminals to endocannabinoid activation, which would indicate that a switch from DSI to DSE might occur when endocannabinoid concentrations at hippocampal synapses attain a certain threshold value. Again, the role of such a switch, if any, is undefined.
Inhibition of glutamatergic neurotransmission by cannabinoid agonists has been documented in a variety of brain structures besides the hippocampus and cerebellum. These include the prefrontal cortex, amygdala, nucleus accumbens, striatum and substantia nigra pars reticulata. Whether such effects reflect the existence of regional DSE-like phenomena is an important question that remains to be addressed.

A similar form of endocannabinoid-dependent LTD can be produced by low-frequency stimulation of cortical fibres that innervate the nucleus accumbens. Despite differences in induction protocols in vitro — one is produced by high-frequency stimulation, the other by low-frequency stimulation — striatal and accumbal LTD could serve complementary functions. For example, they might both contribute to habit formation, a type of striatum-dependent learning that underlies the development of motor skills and is implicated in the pathogenesis of drug addiction. Notably, cannabinoid drugs provoke rats to relapse to drug-seeking behaviour after prolonged periods of abstinence, whereas CB1 antagonists attenuate the relapse induced by drug-associated cues. These findings have provided the rationale for current clinical trials of rimonabant as a treatment for alcohol and tobacco addiction (Box 2).

Hippocampus: a role in cognition? In the hippocampal CA1 field, stimulation protocols that cause long-term potentiation at excitatory synapses onto pyramidal neurons simultaneously produce LTD at adjacent inhibitory synapses (I-LTD). Like striatal LTD, I-LTD might be endocannabinoid-mediated, but its molecular mechanism seems to be remarkably different. According to a current model, glutamate released from excitatory terminals activates metabotropic receptors on dendrites...
of pyramidal neurons, which in turn stimulates 2-AG formation through the DGL pathway. The newly formed endocannabinoid can then depress GABA release by engaging CB receptors on inhibitory nerve endings. How this long-lasting disinhibitory process interacts with other forms of endocannabinoid-dependent plasticity and contributes to the overall effects of cannabinoids on hippocampus-dependent learning will surely be the object of future discussion and experiments.

Missing pieces

A decade of research in the biology of the endocannabinoid system has led to a series of exciting discoveries. We have learned that the brain contains multiple endocannabinoid lipids, and that neurons produce them using membrane constituents as starting material. We have also discovered that these lipids behave differently from traditional transmitters. Rather than being secreted from vesicles, they are released in a non-synaptic manner and combine with cannabinoid receptors located near their sites of synthesis.

Despite this progress, many crucial pieces of the endocannabinoid puzzle are still missing. For example, we need to map the neuronal circuits that produce anandamide and 2-AG, and this requires the formation and expression of endocannabinoid receptors in brain.

By showing that anandamide is produced in and released from brain tissue under physiological conditions, references 14–16 established the role of this compound as an endogenous ligand for cannabinoid receptors.


Olta, S. E. et al. Ether-linked analogue of 2-arachidonyl glycerol (noladin ether) was not detected in the brains of various mammalian species. J. Neurochem. 85, 1374–1381 (2003).


References 41–43 provided the first unequivocal demonstration that endocannabinoids regulate synaptic transmission in the brain.


Together with reference 50, this paper identifies facilitated transport as the first step in anamdoide deactivation and initiates the first anandamide transport inhibitors, AM404 and AM405.


Together with reference 100, this study provided the first indication that an additional brain cannabinoid receptor remains to be cloned.


This paper reports that endocannabinoids can facilitate hippocampal long-term potentiation (LTP) at the single-cell level, although pharmacological administration of cannabinoid agonists inhibits LTP and impairs memory (reviewed in reference 114).


