THE THERAPEUTIC AND RECREATIONAL USE of marijuana has been practised for several centuries, 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. 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. After the cloning and molecular pharmacology became convinced of the existence of cannabinoid receptors, whose action is mimicked by THC?

Are there endogenous ligands selective for cannabinoid receptors? Although Virginia Willis and collaborators in the early 1990s described cannabimimetic activity of a substance purified from porcine brain, this was later named CB1 following the discovery of the CB2 cannabinoid receptor (THC), which was originally known as the peripheral subtype, and the mapping of its distribution in peripheral tissues. Moreover, it has also been confirmed that cannabinoid receptors do exist and mediate most of the effects of THC, and marijuana in central and peripheral tissues. 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 CB1 receptors was identified in porcine brain. 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 hyperthermia. Moreover, anandamide exhibited cross-tolerance to THC in these tests (although only at high concentrations), substituted for THC in drug-discrimination tests, and shared with THC the same G-protein-mediated actions on adenylyl cyclase and Ca2+ channels. 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 CB1 receptor agonist, and a weaker- and shorter-lasting action in vivo of the endogenous metabolite when compared with the most active synthetic compounds. This latter phenomenon, however, is likely to be due to the recently reported rapid degradation of anandamide in vivo. Despite the fact that the existence of other, possibly peptide-like, endogenous ligands of cannabinoid receptors has been suggested, 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-α-linolenoyl-ethanolamide, and 2-arachidonoylglycerol (2-AG), and, analogously 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 system)
REVIEW

Damide is now used only for ethanolamines were previously collectively referred to as anandamides, but the name anandamide is now used only for the selective antagonist of CB1 receptors. Although reverse agonism has been shown for SR141716A, this cannabinoid is used both in vivo and in vitro as a tool to reveal a toxic role by endogenous cannabinoids at some physiological functions.

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 CB1-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 showed that the synthesis and release of anandamide, whose basal levels in brain are low compared to most neurotransmitters, could be stimulated in intact cortical and striatal neurons (but not astrocytes) by treatment with membrane-depolarizing agents such as ionomycin, 4-aminopyridine, kainate and high KCl. Neurons and astrocytes were also both found to re-uptake and hydrolyse anandamide rapidly, resulting in the formation of AA and ethanolamine. The uptake mechanism is mediated by a saturable, selective, temperature-dependent and Na+-independent transporter that facilitates anandamide release, and whose inhibition can potentiate some of anandamide’s actions. Thus, anandamide, like classical neurotransmitters, is released from neurons following membrane depolarization and Ca2+ 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-arachidonyl-phosphatidylethanolamine (NAPE, Fig. 2). This biosynthetic pathway is identical to that proposed in the 1980s for the biosynthesis of saturated and mono-unsaturated N-acyl-ethanolamines which are produced in higher amounts than anandamide by ionomycin-stimulated neurons, and could also apply to the other cannabimimetic fatty acids and amides shown in Fig. 1. The other route proposed for the formation of anandamide is the ATP- and co-enzyme-A-independent condensation of high concentrations of AA and 2-AG through the action of a PI-selective phospholipase C (PI-PLC)32. DAGs may also derive from phosphatidic acid obtained from PLC-independent pathways (shown in dark blue), such as N-acyl-ethanolamine formation or phospholipid remodeling16,17,26. Once synthesized, anandamide is released from neurons through facilitated diffusion. The same carrier probably also mediates anandamide re-uptake by neurons16,22,28. Anandamide and analogues are also capable of diffusing passively through the cell membrane and, in part, anandamide are degraded through the action of the membrane-bound enzyme fatty acid amidohydrolase (FAAH)30,32. Anandamide is also hydrolyzed by other enzymes or partly converted non-enzymatically into the (1S)-isomer of arachidonoyl-PE (AAE); ethanolamine and glyceryl, produced from anandamide and 2-AG, are rapidly re-esterified into membrane phospholipids, and is the part of 2-AG that has diffused into the cell. FAAH can be forced to catalyse the hydrolysis reverse reaction and this could account for the synthesis of anandamide through the condensation of high concentrations of ethanolamine and AA, originally observed in NAPE-containing membrane preparations. Abbreviations: AA, arachidonic acid; AC, adenylyl cyclase; 2-AG, 2-arachidonoylglycerol; NAPE, N-arachidonyl-PE; DAG, diacylglycerol; FAAH, fatty acid amide hydrolase; IP1, inositol-1,4,5-trisphosphate; NAPE-PLD, N-arachidonyl-phosphatidylethanolamine-selective phospholipase D; NAPE, N-arachidonylethanolamine; PA, phosphatidic acid; PL, phosphatidylethanolamine; PKA, protein kinase A; VIP, vasoactive intestinal peptide.
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**Agonists**

1. **NMDA**
   - Membrane-depolarizing stimuli
2. **PE**
3. **ArAPC**
4. **Anandamide precursors**

**Anandamide and 2-AG metabolism**

- **Recycling**
- **Direct esterification into phospholipids**
- **Carrier protein**
- **Anandamide and 2-AG precursors**

**Metabolism**

- **Anandamide**
- **2-AG**
  - **LysoPLD**
  - **de novo synthesis (?) or phospholipid remodelling**

**Release and re-uptake**

- **Ca^{2+}**
- **Electrical activity, membrane-depolarizing stimuli**

**Agonists**

- **VIP**
- **Membrane-depolarizing stimuli**

**Pathways**

- **Agonists**
- **NMDA**
- **PE**
- **ArAPC**
- **Anandamide precursors**

**Pathways**

- **Agonists**
- **NMDA**
- **PE**
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**Pathways**

- **Agonists**
- **NMDA**
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- **ArAPC**
- **Anandamide precursors**
ethanolamine, which was initially substantiated by evidence gained from using cell-free systems29. However, this has never been demonstrated in living cells. Subsequent studies30–33 have managed to charac-

terize 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-di-ara-

chidonoyl-phosphatidylcholine to phosphatidyl-

ethanolamine (Fig. 2). Stimulation of adenylyl-cyclase and cAMP-dependent protein kinase was shown to be a potent activator of this trans-acylase34. The discovery of this enzyme in brain areas containing high levels of anandamide23, for example, the hippocampus, cortex, striatum and cerebellum24, and the observation that its inhibition leads to sup-

pression of NArPE formation by cortical neurons25, support its role in the physiological biosynthesis of this endocannabinoid. It remains to be established, however, how the very low amounts of 1,2-di-ara-

chidonoyl-phosphatidylcholine present in the brain25,26 can sustain the stimulus-induced biosynthe-

sis 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 \(A_\lambda\) release of AA and phospholipid remodelling27.

2-Arachidonoyl-glycerol 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 stimulation28,29. The biosyn-

thesis of 2-AG is preceded by diacylglycerol formation and could follow both phospholipase C (PLC)-
dependent30,31 and independent32 pathways (Fig. 2). An sn-1-selective diacylglycerol lipase would be required in both cases32. Co-release of 2-AG and anandamide, which might result in increased flexibility of their possible neuromodulatory action, has been observed in cortical neurons following ionomycin treatment31,32, but not in electrically stimulated hippocampal slices, where only the formation of 2-AG was reported32. In most cases the arachidonoyl-glycerol, which is 170-times more abundant than anandamide in rat whole brain35, is synthesized in higher amounts than anandamide by stimulated neurons. 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 signal, in view of the weak cannabimimetic properties and for the potentializing effects on endocannabinoid actions that have recently been shown for oleamide36.

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

In the nervous system, endocannabinoids exert most of their pharmacological actions by activating the CB1 receptor, which is preferentially expressed in neurones of specific brain regions37. In transfected cells, the interaction with overexpressed CB1 receptors and the subsequent activation of pertussis toxin-sensi-
tive G-proteins allows anandamide to modulate nega-
tively adenylyl-cyclase and voltage-sensitive Ca\(^{2+}\) channels and to activate the inwardly rectifying K\(^+\) channel38–41 (Fig. 3). Many of these intracellular actions have also been observed in rat brain tissues and cells constitutively expressing CB1 receptors, par-
ticularly: (1) striatal slices, where anandamide inhibits forskolin-induced cAMP formation in rat cor-


tal neurones42, and (2) hippocam-

pal neurones, where anandamide inhibits presynaptic N- and P/Q-type \(\text{Ca}^{2+}\) channels and to activate the inwardly rectifying K\(^+\) channel43,44. In stratal neurones, pertussis toxin treatment unmaska G-protein-mediated linkage of CB1 receptors with adenylyl-cyclase stimulation45. Two recent studies demonstrated that experimental evidence was found for the actual participation of cannabinoid receptors in this anan-
damide and THC effect. 2-Arachidonoyl-glycerol also inhibits forskolin-induced cAMP formation in rat cor-
tical neurones46 and inward \(\text{Ca}^{2+}\) currents in differen-
tiated neuroblastoma \(\times\) glioma NG108-15 cells47.

Interestingly, neither anandamide nor oleamide, neither of which is a strong agonist of \(\text{CB}_1\) receptors, NG108-15 and N18TG2 cells with low nanomolar concentrations of the monoglyceride, but not anan-
damide, leads to a transient rise in intracellular \(\text{Ca}^{2+}\)
levels via a CB1-receptor-mediated, and possibly PLC-mediated, mechanism that has never been described previously for synthetic cannabinoids. The possibility of this effect being due to interaction with a different CB1-receptor-variant, such as that described by Shire et al., has not been investigated. Evidence for the presence of CB1 receptors in astrocytes is still preliminary. 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, was not induced by synthetic cannabinoids and was blocked by pertussis toxin but not by the CB2-receptor antagonist, SR141716A. This antagonist does counteract another pertussis toxin-sensitive effect of anandamide in cortical astrocytes, that is, stimulation of AA release, but only at concentrations five-times higher than anandamide. Thus, CB1 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 I). 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

<table>
<thead>
<tr>
<th>Brain or peripheral region</th>
<th>Neuro-transmitter</th>
<th>Modulatory action</th>
<th>Possible CB-containing cell target</th>
<th>Endo-cannabinoids detected</th>
<th>Metabolic enzymes identified</th>
<th>Possible ultimate effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>Glutamate</td>
<td>Inhibition of release</td>
<td>Glutamatergic CA3 and CA1 neurones</td>
<td>Anandamide, 2-arachidonoyl-glycerol</td>
<td>FAAH, trans-acylase, PI-PLC</td>
<td>Inhibition of LTP</td>
</tr>
<tr>
<td></td>
<td>Acetylcholine</td>
<td>Inhibition of release</td>
<td>Cholinergic neurones of the septohippocampal perforant path in the dentate gyrus</td>
<td>As above</td>
<td>As above</td>
<td>Inhibition of learning and memory</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Glutamate</td>
<td>Inhibition of NMDA receptor-mediated activation of PIQ type Ca²⁺ channel</td>
<td>Glutamatergic granule cells</td>
<td>Anandamide</td>
<td>Anandamide carrier (in granule cells) FAAH, trans-acylase</td>
<td>Inhibition of motor coordination, neuroprotection</td>
</tr>
<tr>
<td>Cortex</td>
<td>Glutamate</td>
<td>As above</td>
<td>Cortical molecular layers</td>
<td>Anandamide, 2-arachidonoyl-glycerol (in cultured cortical neurones)</td>
<td>FAAH, trans-acylase anandamide carrier, PI-PLC, sn-1-DAG-lipase, MAG lipase</td>
<td>Inhibition of memory and motor behaviour, neuroprotection</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Glutamate</td>
<td>Inhibition of release/NMDA receptor-mediated action</td>
<td>Neurones of the dorsal horn</td>
<td>2-arachidonoyl-glycerol (in dorsal root ganglia) PI-PLC, sn-1-DAG-lipase, MAG lipase (dorsal root ganglia)</td>
<td>FAAH, trans-acylase</td>
<td>Inhibition of motor activity, cataleptogenic activity</td>
</tr>
<tr>
<td>Basal ganglia and substantia nigra</td>
<td>GABA</td>
<td>Inhibition of re-uptake</td>
<td>GABAergic striatonigral and striatopallidal neurones</td>
<td>Anandamide (in the striatum)</td>
<td>FAAH (in the substantia nigra and globus pallidus) trans-acylase (in the striatum) anandamide carrier (in cultured striatal neurones)</td>
<td>Inhibition of locomotor activity, cataleptogenic activity</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Inhibition of synthesis/release action</td>
<td>Dopaminergic nigrostriatal neurones</td>
<td>As above</td>
<td>As above</td>
<td>FAAH</td>
<td>Inhibition of locomotor activity</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Potentiation</td>
<td>As above</td>
<td>Dopaminergic neurones of the tubero-infundibular system</td>
<td>No evidence</td>
<td>FAAH</td>
<td>Inhibition of prolactin release from the pituitary</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>Dopamine</td>
<td>Potentiation</td>
<td>Dopaminergic neurones of the tubero-infundibular system</td>
<td>No evidence</td>
<td>No evidence</td>
<td>Inhibition of smooth muscle contractions</td>
</tr>
<tr>
<td>Parasympathetic prejunctional fibres</td>
<td>Acetylcholine</td>
<td>Inhibition of release</td>
<td>Bladder, myenteric neurones</td>
<td>No evidence</td>
<td>No evidence</td>
<td>Unknown</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>Noradrenaline</td>
<td>Inhibition of release (via NO release from renal endothelial cells)</td>
<td>Renal endothelial cells</td>
<td>Anandamide (in renal endothelial and mesangial cells)</td>
<td>FAAH (in renal endothelial and mesangial cells)</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Inhibition of release from postganglionic fibres</td>
<td>Superior cervical ganglion</td>
<td>Anandamide (from macrophages)</td>
<td>No evidence</td>
<td>Hypotension, bradycardia</td>
<td></td>
</tr>
</tbody>
</table>

**Other neuromodulatory actions have been suggested for Δ⁹-9-tetrahydrocannabinol (THC) (see Ref. 55 for a review), but there is no evidence as yet that they also occur with the endocannabinoids. These are: inhibition of glutamate release from the subthalamic nucleus, from the cerebellar granule cells that stimulate Purkinje cells, and from the paraglomerular grey; 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 facilitation of α₁-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.**

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postsynaptic depolarization and of action potential generation⁶⁹,⁷⁰. Presynaptic inhibition of adenylyl cyclase could also lead to activation of A-type K⁺ channels, as shown for THC in cultured hippocampal cells⁶⁹, thus further inhibiting transmitter release. Anandamide can, therefore, inhibit AMPA/kainate-receptor-mediated
neurotransmission in hippocampal neurones by acting at the presynaptic level66. 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 hippocampus67. These data could provide a molecular explanation for the CB1-receptor-mediated impairment of working memory in rodents by anandamide68, 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 transfor- mation of long-term potentiation to long-term depression69. 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, synthesizes monosaccharidonylglycerols), 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 endorphines70, and the potent hyperalgesic properties of SR141716A and of CB1-receptor-mRNA antisense oligonucleotides, which strongly support the existence of a tonic inhibition of thermal nociception by spinal endocannabinoids, are reversed by NMDA-receptor antagonists71. Finally, anandamide inhibition of NMDA receptor currents is blocked by the antagonist SR141716A (Refs 49, 50). What is behind the endogenous cannabinoid system? Six years of intensive research on endocannabi- noids 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 intake72, inhibition of anxiety-like responses73 and decrease of arousal74 in rodents. Moreover, anandamide has been shown to inhibit aggressiveness in singly housed mice75. Therefore, on the basis of the findings reviewed here, it might be intriguing to speculate that the endocannabinoids have a general function in 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 cannabinoids or N-acylphosphatidylethanolamines, or both, has been suggested by the following findings: (1) an antioxida-

tive inhibition of NMDA receptor-mediated Ca2+


induction of excitotoxicity79 and (3) increased levels of these compounds following gluta-

tamine stimulation or cell injury.80 Thus, ‘relax, eat, sleep, forget and protect’ might be some of the

messages that are produced by the actions of endo-


cannabinoids, alone or in combination with other mediators. However, several aspects of endocanabi-

noid 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


psychologists to evaluate critically this or other hypotheses that relate to the physiological signifi-


cance of the endogenous cannabinoid system.


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