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The endocannabinoid system as a target for therapeutic drugs
Daniele Piomelli, Andrea Giuffrida, Antonio Calignano and Fernando Rodriguez de Fonseca

Cannabinoid receptors, the molecular targets of the cannabis constituent Δ⁹-tetrahydrocannabinol, are present throughout the body and are normally bound by a family of endogenous lipids – the endocannabinoids. Release of endocannabinoids is stimulated in a receptor-dependent manner by neurotransmitters and requires the enzymatic cleavage of phospholipid precursors present in the membranes of neurons and other cells. Once released, the endocannabinoids activate cannabinoid receptors on nearby cells and are rapidly inactivated by transport and subsequent enzymatic hydrolysis. These compounds might act near their site of synthesis to serve a variety of regulatory functions, some of which are now beginning to be understood. Recent advances in the biochemistry and pharmacology of the endocannabinoid system in relation to the opportunities that this system offers for the development of novel therapeutic agents will be discussed.

Since the discovery of the first cannabinoid receptor 12 years ago, important advances have been made in several areas of cannabinoid pharmacology. Endocannabinoid compounds and their pathways of biosynthesis and inactivation have been identified, and the molecular structures and anatomical distribution of cannabinoid receptors have been investigated in detail. Pharmacological agents that interfere with various aspects of the endocannabinoid system have been developed, and pathophysiological circumstances in which this system might be active have begun to emerge. The manner in which these discoveries might impact our understanding of endocannabinoid signaling and help unlock its potential for developing novel therapeutic agents will be discussed.

Endocannabinoids

The two endocannabinoids isolated so far – anandamide and 2-arachidonylglycerol (2-AG) – are lipid in nature but differ from amino acid, amine and peptide transmitters in ways other than just their chemical structures. Classical and peptide transmitters are synthesized in the cytosol of neurons and stored in synaptic vesicles, from where they are secreted by exocytosis following excitation of nerve terminals by action potentials. By contrast, anandamide and 2-AG can be produced upon demand by receptor-stimulated cleavage of membrane lipid precursors and released from cells immediately after their production.

Anandamide can be produced from the hydrolysis of an N-acylated species of phosphatidylethanolamine (PE) – arachidonoyl PE, a process catalysed by phospholipase D (PLD) (Fig. 1). The stimulation of neurotransmitter receptors appears to play a determinant role in initiating this reaction, as indicated by the finding that anandamide release in the stratum is strongly enhanced by activation of dopamine D₂ receptors. Once released, anandamide can act on cannabinoid receptors or accumulate back into cells via an energy- and Na⁺-independent transport system. The selectivity of this system for anandamide has been documented but its molecular structure remains uncharacterized. Inside cells, anandamide can be catalytically hydrolysed by an arachidonylacylhydrolase, whose gene has been cloned (Fig. 1).

The most likely route of 2-AG biosynthesis involves the same enzymatic cascade that catalyses the formation of the second messengers inositol (1,4,5)-triphosphate and 1,2-diacylglycerol (DAG) (Fig. 2). Phospholipase C (PLC), acting on phosphatidylinositol (4,5)-bisphosphate, generates DAG, which is converted to 2-AG by DAG lipase. 2-AG might also be synthesized by the hydrolysis of lysophospholipids or triacylglycerols. Regardless of the mechanism involved, 2-AG formation can be triggered by neural activity or by occupation of membrane receptors. Following its release, 2-AG can be taken up by cells via the anandamide transport system and hydrolysed by an unknown monoaoylglycerol lipase activity (Fig. 2).

Thus, anandamide and 2-AG can be released from neuronal and non-neuronal cells when the need arises, utilizing analogous but distinct receptor-dependent pathways. The non-synaptic release mechanisms and short life spans of anandamide and 2-AG suggest that these compounds might act near their site of synthesis to regulate the effects of primary messengers, such as neurotransmitters and hormones.

Inhibitors of anandamide inactivation

Drugs that block the formation or inactivation of anandamide and 2-AG should help identify the physiological functions of these compounds and might be beneficial in disease states in which regulation of endocannabinoid levels might produce more selective responses than those elicited by cannabinoid receptor ligands. Although this area of pharmacology is still largely unexplored, inhibitors of the two main steps of anandamide disposition (membrane transport and intracellular hydrolysis) have recently become available.

Anandamide transport is inhibited by the compound AM404 (Figs 1,3). This drug potentiates various responses elicited by exogenous anandamide and interacts very poorly with cannabinoid CB₁ receptors. For example, AM404 enhances anandamide-induced hypotension without producing...
direct vasodilatory effects16. Furthermore, when applied alone, AM404 decreases motor activity17,18 and elevates the levels of circulating anandamide (A. Giuffrida et al., unpublished).

However, AM404 can accumulate in cells where it might reach concentrations that are sufficient to inhibit anandamide amidohydrolase9 (M. Beltramo and D. Piomelli, unpublished).

Anandamide amidohydrolase is blocked reversibly by transition state analogs such as arachidonyltrifluoromethylketone (ATFMK), which might act by forming a stable inter- 

mediate with a serine residue at the enzyme active site18

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role of CB1 receptors in mediating central cannabinoid effects; 

issues that might be relevant to the use of cannabinoid agents 

ated by the GPCR-kinase– 

receptor desensitization. This process, which might be medi-

bated by a variety of compounds including the fatty acid sulfonil 

dioxide. AM374 (Ref. 18) (Figs 1,3). AM374, one of the 

most potent anandamide amidohydrolase inhibitors identified 

thus far, potentiates anandamide responses in vitro and in vivo, 

but its specificity is limited by a relatively high affinity for 

CB1 receptor19.

Cannabinoid receptors

The two cannabinoid receptor subtypes characterized so far, 

CB1 and CB2, belong to the superfamily of G-protein-coupled 

membrane receptors (GPCRs)2,20. Their molecular and phar-

macological properties have recently been reviewed21. Three 

issues that might be relevant to the use of cannabinoid agents 

in medicine will be discussed: (1) the apparently exclusive 

role of CB1 receptors in mediating central cannabinoid effects; 

(2) the rapid tolerance that results from repeated cannabinoid 

administration; and (3) the possible existence of multiple 

cannabinoid receptors in peripheral tissues.

Although CB1 receptors are expressed throughout the body, 

they are particularly abundant in the CNS where, despite a 

great deal of effort, no other cannabinoid receptor subtype 

has yet been found. This unusual situation – most neurotrans-

mitters act on multiple CNS receptors – accords with data 

that indicate that a single pharmacological site accounts for all 

central effects of cannabinimetic drugs, whether therapeuti-

cally favorable (e.g. analgesia) or harmful (e.g. dysphoria and 

amnesia). Consequently, although potent CB1 receptor ag-

onists have been available for some time (Table 1), the thera-
pic development of these compounds has been very limited.

Given this situation, how might centrally active cannabinoid 

agents that are more selective than those currently available 

be developed? One possibility is to target the mechanisms of 

dendroanandimetic inactivation. Blocking such mechanisms 

might cause an activity-dependent accumulation of anao-

damide and 2-AG at their sites of release, which might in turn 

result in a more localized activation of cannabinoid receptors 

than that elicited by direct receptor agonists.

Another important issue that should be considered in the 

development of cannabinimetic agonists for therapeutic use 

is receptor desensitization. This process, which might be medi-

ated by the GPCR-kinase–β-arrestin pathways22,23, causes a 

pharmacological tolerance that limits the prolonged use of 

cannabinoid receptor agonists. Partial agonists might offer a 

cue as to how to circumvent this obstacle. Evidence indicates 

that the CNS contains a large number of CB1 receptors24; thus, 

partial CB1 receptor agonists, which are expected to cause 

less receptor desensitization than full agonists, might produce 

adequate therapeutic responses with diminished tolerance 

liability.

Although CB1 receptors are thought to mediate the effects 

cannabinoid receptor agonists in the CNS, several periph-

eral effects of cannabinimetic drugs might only depend par-

tially on CB1 receptor activation. The high expression of CB2 

receptor in B cells and natural killer cells suggests that this 

subtype contributes to the potential immunosuppressant and 

anti-inflammatory effects of cannabinoids25. Additional tests 

of this hypothesis will be facilitated by the recent availability 

of selective CB1 receptor agonists and antagonists (Table 1).
functions and strategies

The endocannabinoid system might serve important regulatory functions in physiological processes; thus, cannabinoid agents might prove useful in the treatment of pathological conditions that are associated with such processes. Exhaustive evaluations of the medicinal potential of cannabis and its derivatives in other therapeutic areas can be found elsewhere.

Modulation of pain

Cannabinoids strongly reduce pain responses by interacting with CB1 receptors in brain, spinal cord and peripheral sensory neurons (Fig. 4). Brain sites that participate in cannabinoid-induced analgesia include the amygdala, thalamus, septor colloidalus, periaqueductal gray and rostral ventromedial medulla. In the spinal cord, CB1 receptors are found in the dorsal horn and lamina X (Ref. 21), where they are located on intrinsic spinal neurons, nerve terminals of afferent sensory neurons and terminals of different supraspinal neurons (Fig. 4). CB1 receptors are also expressed in the dorsal root ganglia by a subset of small- and large-diameter sensory neurons that contain the pain-stimulating peptides, substance P and C-fibers (Ref. 22). The clinical impact of these advances is still modest but worth noting. Since a previous literature review (Ref. 23), new studies have documented the analgesic effects of CB1 receptor agonists in humans (for example, Ref. 24), providing additional impetus for a re-evaluation of the endocannabinoid system as a target for analgesics.

Neuropathic pain

Cannabinoids are potent in alleviating two hallmarks of neuropathic pain: allodynia (pain from non-noxious stimuli) and hyperalgesia (increased sensitivity to noxious stimuli). Indeed, in a rat model of neuropathic pain (constriction injury of the sciatic nerve), the CB1 receptor agonist WIN552122 attenuates such responses at doses that do not cause overt side-effects (Ref. 25). In this model, SR141716A enhances the sensitivity to mechanical stimuli applied to the paw contralateral to the inflammatory focus, which suggests that...
inflammation can be accompanied by an increased cannabinoid activity that can be unmasked by the CB1 receptor antagonist41. Furthermore, the peripheral administration of formalin stimulates anandamide release in the periaqueductal gray, a brain region involved in pain control42. Whether CB1 receptor function and/or endocannabinoid levels are changed in neuropathic pain is unknown. If this syndrome is accompanied by a hypersensitivity of CB1 receptors in injured tissues, partial CB1 receptor agonists could alleviate pain at doses that might exert few undesirable effects and produce little tolerance. By contrast, if neuropathic pain is associated with elevated endocannabinoid release, drugs that interfere with the inactivation of these substances might offer an alternative to direct CB1 receptor agonists. Elucidating the alterations in endocannabinoid function associated with neuropathic pain should be instrumental to define the value of these strategies.

Peripheral pain

The finding that cannabinoid receptor agonists can alleviate pain by acting at peripheral CB1 receptors35,36 has both theoretical and practical ramifications. Theoretically, this observation emphasizes the notion that nociceptive signals can be modulated at the first stage of neural processing by a peripheral ‘gate’ mechanism in which endogenous cannabinoid lipids can act in concert with opioid peptides43. Practically, it points to the possibility of achieving an effective control of peripheral pain without causing the psychotropic effects that follow the recruitment of brain CB1 receptors.

The antinociceptive effects of palmitylethanolamide add a new dimension to this hypothesis35. Palmitylethanolamide is produced in tissues through an enzymatic route similar to that of anandamide synthesis6. When administered as a drug, palmitylethanolamide potently reduces peripheral pain through a mechanism that is synergistic with anandamide and is blocked by the CB2 receptor antagonist SR144528 (Ref. 35). However, palmitylethanolamide does not interact with the CB2 receptor (whose gene has been cloned), which suggests that the compound might produce its analgesic effects by activating an as-yet uncharacterized CB2-like receptor35.

Regulation of glutamate transmission

CB1 receptor agonists inhibit both glutamatergic neurotransmission and long-term potentiation (LTP)44, a model of glutamate-dependent synaptic plasticity. These effects, which can be triggered by activation of presynaptic CB1 receptors and mediated by inhibition of glutamate release, might reflect a fundamental role of the endocannabinoid system in the regulation of excitatory neurotransmission (Box 1). Two lines of evidence suggest that this might be the case. In hippocampal slices, electrical stimulation of glutamate-releasing fibers enhances 2-AG formation, a response that might depend on the activation of NMDA receptors12 (N. Stella and D. Piomelli, unpublished). In the same preparation, exogenous 2-AG potentiates the induction of LTP by activating CB1 receptors12, which indicates that neurally released 2-AG might act as a negative feedback signal regulating transmission at glutamate synapses (Box 1). Whether or not this hypothesis turns out to be correct, the interaction between cannabinoid and glutamate-mediated signaling opens important perspectives for therapy.

Brain ischaemia

A primary pharmacological approach to ischaemic brain injury aims to arrest excitotoxicity, the neuronal death triggered by glutamate via activation of Ca2+-permeable ionotropic receptors45. On the basis of their ability to reduce excitotoxicity

### Table 1. Cannabinoid receptor ligands

<table>
<thead>
<tr>
<th>Receptor subtype</th>
<th>CB1</th>
<th>CB2</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonselective agonists</td>
<td>HU210 (tricyclic cannabinoid)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CP55940 (bicyclic cannabinoid)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WIN55218 (aminoalkylindole)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACPA</td>
<td>J Neurochem 79, 595 (1999)</td>
<td>57,58</td>
</tr>
<tr>
<td></td>
<td>ACEA</td>
<td>J Neurochem 79, 595 (1999)</td>
<td>57,58</td>
</tr>
</tbody>
</table>

Abbreviations: ACPA, arachidonylcyclopropylamide; ACEA, arachidonyl-2-chloroethylamide.
CB1 receptor antagonist SR141716A, which has little effect on motor activity when administered alone, potentiates the motor hyperactivity produced by the D2 receptor agonist quinpirole. Third, D2 and CB1 receptor agonists produce opposing behavioral responses after injection into the basal ganglia. These and other findings suggest that anandamide might modulate dopamine-induced facilitation of psychomotor activity. In further support of this hypothesis, disruption of the gene encoding the CB1 receptor profoundly affects motor control, decreasing locomotor activity.

Movement disorders

The recommendation by the Institute of Medicine that studies be conducted to test the hypothesis that cannabinoids play an important role in movement disorders is justified by a significant body of experimental and clinical evidence. Preclinical studies have focused on the possible application of CB1 receptor agonists in the management of dyskinesias that accompany the treatment of Parkinson’s disease with L-DOPA. Clinical investigations have been primarily concerned with the ability of CB1 receptor agonists to alleviate spasticity in various conditions and tics in Tourette’s syndrome. In particular, a recent double-blind trial has demonstrated significant improvements in tics and obsessive compulsive behaviors following administration of the oral cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC) to 12 Tourette patients (K. Müller-Vahl et al., unpublished). However, these improvements were accompanied in five patients by mild side-effects that included fatigue, dizziness and euphoria.

Psychoses

There is a general consensus that heavy cannabis abuse can precipitate psychotic episodes in individuals with an underlying schizophrenic condition. This idea, which is supported

Box 1. A hypothetical model of the role of endocannabinoid signaling in glutamate neurotransmission

Flux of external Ca2+ through activated NMDA receptor channels can stimulate phospholipase C (PLC) (Fig. I), which initiates 2-acyl-glycerol (2-AG) formation via diacylglycerol (DAG) lipase (N. Stella and D. Piomelli, unpublished). Newly formed 2-AG can activate cannabinoid CB1 receptors on presynaptic nerve terminals, which might in turn reduce glutamate release and decrease synaptic strength. It is important to note that several regions of the CNS, CB1 receptors are located on axon terminals of GABA-containing neurons, where they might be linked to inhibition of GABA release. Moreover, excitatory effects of cannabinoids mediated by changes in postsynaptic ion conductances have also been reported. Thus, the net effect of CB1 receptor activation might be more to produce a functional reconfiguration of neuronal networks than just to blunt glutamate-mediated transmission.

Selected references

- Stubbs, B. et al. (1998) Inhibition of GABAergic synapses by cannabinoids in rat corpus striatum. Neuroscience 85, 395–405

- Katona, I. et al. (1999) Inhibition of GABAergic synapses by cannabinoids in rat corpus striatum. Neuroscience 85, 395–405
by substantial epidemiological evidence\(^5\), instigated an ongoing clinical trial of the CB1 receptor antagonist SR141716A in schizophrenic patients. Yet, on examining the basis of cannabis-precipitated psychosis, consideration should also be given to CB1 receptor desensitization and to the fact that this process can have repercussions that go beyond behavioral tolerance. One such repercussion is an exacerbated response to the psychostimulant, D-amphetamine. In animals, D-amphetamine increases motor activity and stereotypies, an effect that depends on dopamine receptor activation and is blocked by D2 receptor antagonists. Because D-amphetamine can also trigger psychotic episodes in schizophrenics, the behavioral response to D-amphetamine in animals is often used as a screening test for antipsychotic medications. The stimulation of stereotyped movements elicited by D-amphetamine is blocked by acute administration of \(\Delta^2\)-THC, but this same stimulation is increased in animals that have been made tolerant to cannabinoids by repeated injections of \(\Delta^2\)-THC (Ref. 53). Thus, CB1 receptor activation might counterbalance stimulation of dopamine-containing neurons, whereas CB1 receptor inactivation might enhance such stimulation. In this framework, cannabis use by schizophrenics might be interpreted as a misguided attempt to obtain relief from psychotic symptoms\(^6\), which might in turn facilitate a psychotic episode when CB1 receptors become desensitized.

The ability of \(\Delta^2\)-THC to reduce tics in Tourette’s syndrome and to inhibit to D-amphetamine-induced stereotypy suggests that CB1 receptor agonists might be therapeutically useful to alleviate the symptoms of dopamine hyperactivity associated with many neuropsychiatric conditions. However, the psychotomimetic effects produced even by low doses of \(\Delta^2\)-THC in Tourette patients and the possible impact of CB1 receptor desensitization underscore the need to investigate a wider variety of cannabinoid agents (e.g. inhibitors of endocannabinoid inactivation) in animal models of motor disorders and psychosis. For example, evidence suggests that the anandamide transport inhibitor AM404 can normalize motor activity in genetically hyperactive rats without causing overt cannabinaminetic effects\(^7\).

**Concluding remarks**

The image of the endocannabinoid system that gleams through the studies summarized in this review is that of a modulatory complex that is parallel, in its varied functional roles, to the opioid system but analogous, for its biochemical properties, to other lipid mediators such as the eicosanoids. It would be surprising if such a prominent signaling system, which gives every indication of serving key physiological functions in the CNS and in peripheral tissues, will fail to prompt the development of new medicines in the not too distant future.

**Selected references**

REVIEW

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48 Sulada-Palit, M.C. et al. (1998) Effects of intracerebroventricular cannabinoid on rat interactions with the dopaminergic system. Apoace 30, 221–226

Chemical names

<table>
<thead>
<tr>
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<td>WIN55482</td>
<td>2(3S,5R)-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-5-(4-chlorophenyl)-1-(4-methylbenzyl)-1-[(1R)-endo-1.3.3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chlorophenyl)-1-(2,4-dimethylphenyl-1H-pyrrol-3-yl)propan-2-ol (WIN552122)</td>
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<td>SR141716A</td>
<td>[1S,1R,2S,3S,4S]-endo-1.3.3-trimethylbicyclo[2.2.1]heptan-2-yl)-5-(4-chlorophenyl)-1-(4-methylphenyl)-1H-pyrrol-3-carboxamide (SR141716A)</td>
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<td>SR144528</td>
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