The endocannabinoid system as a target for therapeutic drugs

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Cannabinoid receptors, the molecular targets of the cannabinoid 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) N-arachidonoyl PE, a process catalysed by phospholipase D (PLD) (Fig. 1). The stimulation of neurotransmitter receptors plays a pivotal role in initiating this reaction, as indicated by the finding that anandamide release in the striatum 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 amidohydrolase, 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)-trisphosphate 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 monoaerolipid 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 effects. Furthermore, when applied alone, AM404 decreases motor activity 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 amidohydrolase (M. Bolton and D. Piomelli, unpublished).

Anandamide amidohydrolase is blocked reversibly by transition state analogs such as arachidonyltrifluoromethylketone (ATFMK), which might act by forming a stable intermediate with a serine residue at the enzyme active site (Fig. 3). Moreover, irreversible inhibition can be achieved with a variety of compounds including the fatty acid sulfonylesters, AM577 (Ref. 18) (Fig. 1.3). AM577, one of the most potent anandamide amidohydrolase inhibitors identified thus far, potentiates anandamide responses in vivo and in vitro, but its specificity is limited by a relatively high affinity for CB1 receptor (1).

Cannabinoid receptors

The two cannabinoid receptor subtypes characterized so far, CB1 and CB2, belong to the superfamily of G-protein-coupled membrane receptors (GPCRs). Their molecular and pharmacological properties have recently been reviewed. 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 neurotransmitters act on multiple CNS receptors – accords with data that indicate that a single pharmacological site accounts for all central effects of cannabinoid drugs, whether therapeutically favorable (e.g. analgesia) or harmful (e.g. dysphoria and amnesia). Consequently, although potent CB1 receptor agonists have been available for some time (Table 1), the therapeutic 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 endocannabinoid inactivation. Blocking such mechanisms might cause an activity-dependent accumulation of anandamide and 2-AG at their sites of release, which might influence 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 cannabinoid agonists for therapeutic use is receptor desensitization. This process, which might be mediated by the GPCR-kinase-β-arrestin pathway, causes a pharmacological tolerance that limits the prolonged use of cannabinoid receptor agonists. Partial agonists might offer a clue as to how to circumvent this obstacle. Evidence indicates that the CNS contains a large number of CB1 receptor subtypes, 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 of cannabinoid receptor agonists in the CNS, several peripheral effects of cannabinoid drugs might only depend partially on CB1 receptor activation. The high expression of CB1 receptors in B cells and natural killer cells suggests that this subfamily contributes to the potential immunomodulatory and anti-inflammatory effects of cannabinoids. 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

Cannabinoid drugs 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, 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 ofafferent sensory neurons and terminals of efferent supraspinal neurons (Fig. 4). CB₂ 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 calcitonin gene-related peptide (CGRP). Although quantitatively small, the presence of CB₂ receptors on CGRP-containing neurons appears to be functionally significant because CB₂ receptor agonists effectively reduce CGRP release from dorsal horn tissue. Immunohistochemical experiments suggest that CB₂ receptors are present not only on central terminals of primary sensory afferents, but also on their peripheral counterparts. In agreement with these findings, local applications of CB₂ receptor agonists to skin reduce the responses to formalin and other irritants.

The clinical impact of these advances is still modest but worth noting. Since a previous literature review, new studies have documented the analgesic effects of CB₁ receptor agonists in humans (for example, Ref. 30), providing additional impetus for a re-evaluation of the endocannabinoid system as a target for analgesic drugs.

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 antagonist WIN552122 attenuates such responses at doses that do not cause overt side-effects.

In this model, the CB₁ receptor antagonist SR141716A reverses the analgesic response to WIN552122 and exacerbates pain behaviors when administered alone. One possible explanation for the pain-inducing effects of SR141716A is that nerve injury might be associated with an increase in endocannabinoid levels and/or a sensitization of CB₁ receptors.

Plastic modifications in endocannabinoid signaling during persistent pain can also be inferred from experiments conducted in a rat model of inflammation (injection of Freund’s adjuvant into the paw). 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 cannabinergic activity that can be unmasked by the CB1 receptor antagonist. Furthermore, the peripheral administration of formalin stimulates anandamide release in the periaqueductal gray, a brain region involved in pain control.

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 be beneficial to 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 receptors 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 peptides. 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 hypothesis. Palmitylethanolamide is produced in tissues through an enzymatic route similar to that of anandamide synthesis. 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 receptor.

Regulation of glutamate transmission
CB1 receptor agonists inhibit both glutamatergic neurotransmission and long-term potentiation (LTP), 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. 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 receptors (N. Stella and D. Piemelli, unpublished). In the same preparation, exogenous 2-AG prevents the induction of LTP by activating CB1 receptors, 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.

| Table 1. Cannabinoid receptor ligands |
|--------------------------------------|
| Receptor subtype* | CB1 | CB2 | Refs |
|-------------------|-----|-----|-----|
| Nonspecific agonists | HU210 (tricyclic cannabinoid) | 54 |
| CP55940 (bicyclic cannabinoid) | 54 |
| WIN55,212 (omega-6) | 54 |
| Specific agonists | Methanandamide | 55,56 |
| CPA | 57,58 |
| ACEA | 57,58 |
| SU14683 (tricyclic cannabinoid) | 59 |
| Selective antagonists | SR141716A | 60,61 |

Abbreviations: ACPA, arachidonylcyclopropylamide; ACEA, arachidonyl-2-chloroethylamide.
CB1 receptor antagonist SR141716A, which has little effect on 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.

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

Flux of external Ca2+ through activated NMDA receptor channels can stimulate phospholipase C (PLC). (Fig. 1) which initiates 2-arachidonylglycerol (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 increase 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.

Regulation of dopamine transmission

CB1 receptors are densely expressed in the basal ganglia and cortex, CNS regions that are critical for movement control. This distribution provides an anatomical substrate for functional interactions between the endocannabinoid system and ascending dopaminergic pathways. Several observations suggest that these interactions might indeed occur. First, in the striatum of freely moving rats anandamide release is stimulated by activations of dopamine D2 receptors. Second, the 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-dihydroxyphenylalanine (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 behavior following administration of the oral cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC) to 12 Tourette patients (K. Muller-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...
by substantial epidemiological evidence, 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, τ-ampetamine. In animals, τ-ampetamine increases motor activity and stereotypies, an effect that depends on dopamine receptor activation and is blocked by D2 receptor antagonists. Because τ-ampetamine can also trigger psychotic episodes in schizophrenics, the behavioral response to τ-ampetamine in animals is often used as a screening test for antipsychotic medications. The stimulation of stereotyped movements elicited by τ-ampetamine is blocked by acute administration of Δ9-THC, but this same stimulation is increased in animals that have been made tolerant to cannabinoids by repeated injections of Δ9-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, which might in turn facilitate a psychotic episode when CB1 receptors become desensitized. The ability of Δ9-THC to reduce tics in Tourette’s syndrome and to inhibit τ-ampetamine-induced stereotypies 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 Δ9-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 modular 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. The functions in the CNS and in peripheral tissues, will fail to prompt the development of new medicines in the not too distant future.

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Chemical names

C25H48O4: (2S)-5-(3-allyloxy-4-(1,3-dimethyl-6,6-dimethoxy-9-methylene-6,6-dihydro-1,4-benzoxazin-3-yl)[2,3-dihydro-5-methyl-3-[4-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.

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C25H48O4: (2S)-5-(3-allyloxy-4-(1,3-dimethyl-6,6-dimethoxy-9-methylene-6,6-dihydro-1,4-benzoxazin-3-yl)[2,3-dihydro-5-methyl-3-[4-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.

C25H48O4: (2S)-5-(3-allyloxy-4-(1,3-dimethyl-6,6-dimethoxy-9-methylene-6,6-dihydro-1,4-benzoxazin-3-yl)[2,3-dihydro-5-methyl-3-[4-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.

C25H48O4: (2S)-5-(3-allyloxy-4-(1,3-dimethyl-6,6-dimethoxy-9-methylene-6,6-dihydro-1,4-benzoxazin-3-yl)[2,3-dihydro-5-methyl-3-[4-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.

C25H48O4: (2S)-5-(3-allyloxy-4-(1,3-dimethyl-6,6-dimethoxy-9-methylene-6,6-dihydro-1,4-benzoxazin-3-yl)[2,3-dihydro-5-methyl-3-[4-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide.