Preclinical science regarding cannabinoids as analgesics: An overview

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Herbal cannabis has been used for centuries for medicinal and recreational purposes, but it has only been in the past 40 years that scientists have been able to elucidate the molecular basis of cannabinoid action.

Modern pharmacology of cannabinoids began in 1964 with the isolation and partial synthesis of delta-9-tetrahydrocannabinol, the main psychoactive agent in herbal cannabis. Since then, potent antinociceptive and antihyperalgesic effects of cannabinoid agonists in animal models of acute and chronic pain; the presence of cannabinoid receptors in pain-processing areas of the brain, spinal cord and periphery; and evidence supporting endogenous modulation of pain systems by cannabinoids has provided support that cannabinoids exhibit significant potential as analgesics. The present article presents an overview of the preclinical science.

Key Words: Cannabinoid opioid interactions; Cannabinoid receptors; Cannabinoids; Chronic pain; Endocannabinoids

Distribution of cannabinoid receptors

CB1 receptors are found in particularly high concentrations in the central nervous system; indeed, CB1 receptors are 10 times more abundant than mu opioid receptors in the brain (11). CB2 receptors are also present in peripheral neurons and in non-neuronal tissues. The distribution of cannabinoid receptors has been examined by several methods (12-14). High levels of CB1 receptors have been found in the hippocampus, basal ganglia, hypothalamus, cerebellum, areas of the cerebral cortex and the nucleus accumbens, with implications for memory, coordination, feeding, higher cognitive function and reward. Most important for pain are moderately abundant concentrations located within the periaqueductal gray (PAG) of the midbrain, the rostral ventrolateral medulla (RVM), superficial layers of the spinal dorsal horn and dorsal root ganglion, from which they are transported to the peripheral and central terminals of the primary afferent neuron (Figure 2). These locations are important in descending pain modulation, spinal processing of pain and peripheral pain perception. Additional areas include the hypothalamus and the pituitary gland (temperature regulation, endocrine and reproductive function), the
amygdala (emotional response and fear), the brainstem (arousal) and the nucleus of the solitary tract (nausea and vomiting) (2,12,15-21) (Figure 2).

There are low levels of cannabinoid receptors in brainstem cardiopulmonary centres, which probably accounts for the high safety margin of the cannabinoids (5). The identification of receptors in the areas described above is consistent with the behavioural effects produced by cannabinoids.

The first CB2 receptors were cloned not from brain, but from a human immune cell line (12); thus, it was apparent from the beginning that the cannabinoid system extended beyond the nervous system. Since that time, studies have demonstrated the presence of CB2 receptors throughout the immune system (22,23).

This work has established the current model for cannabinoid receptors, with CB1 primarily located in brain and associated structures such as the pituitary gland and peripheral nervous tissues, and CB2 primarily located in the reproductive and immune systems (2,23). Recently, CB2 receptor-like immunoreactivity has been described in the rat brain in neuronal patterns supporting possible broader central nervous system roles for the CB2 receptor (24).

Endocannabinoids

Until the end of the 20th century, only two major endocannabinoids, anandamide (N-arachidonoyl-ethanolamine [AEA]) and 2-arachidonylglycerol (2-AG) had been discovered (25-27). Since then, additional endocannabinoids have been identified (28). These include noladin ether, virodhamine (O-arachidonoyl-ethanolamine) and N-arachidonoyl dopamine (NADA) (29-31), as well as others that are in the process of being identified.

To qualify as an endocannabinoid, the agent must exhibit activity at cannabinoid receptors. The endocannabinoids vary in their activity at the receptor depending on the type of intracellular event measured (32). AEA, NADA and noladin are more selective for CB1, virodhamine appears to prefer CB2, and 2-AG is equipotent for both CB1 and CB2 (28). In addition to CB1 agonist activity, AEA binds to the vaniloid receptor (29). NADA also exhibits activity at vaniloid receptors (now called transient receptor potential vanilloid 1 receptors) and appears to be pronociceptive (28). Palmitoylethanolamide (PEA) is not strictly an endocannabinoid, but has cannabimimetic properties, including analgesic effects, which in vivo are antagonized by the CB2 receptor antagonist SR144528 (7) (Table 1).

Biosynthesis and inactivation of endocannabinoids

Endocannabinoids are biosynthesized via a phospholipid-dependent pathway (Figure 3). The metabolic pathway for AEA and 2-AG have been identified; the detailed biosynthesis of the more recently discovered endocannabinoids is currently being worked out (28). The balance of evidence supports that AEA and 2-AG are synthesized and released on demand following physiological and pathological stimuli such as neuronal depolarization and the presence of bacterial lipopolysaccharides, possibly depending on calcium-dependent remodelling of phospholipid precursors. After biosynthesis, AEA and 2-AG are immediately released into the extracellular space. The release, disposition and potential recycling of endocannabinoids is not well understood. Research groups are pursuing various lines of inquiry, including identification of a putative transporter, uptake via caveolin-mediated endocytosis and passive diffusion. Inactivation of AEA and 2-AG occurs via fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase, respectively (4,6,28,33).

CANNABINOID PHARMACOLOGY, MECHANISMS AND SITES OF ACTION

There are several chemical classes of cannabinoid receptor agonists. These are the ‘classical’ cannabinoid ∆9-THC, the ‘nonclassical’ cannabinoid CP55,940, the aminoalkylindole WIN55,212-2, the ‘eicosanoid’ cannabinoid AEA, and additional fatty acid ethanolamides and esters that act as endocannabinoids (2,4). As with the endocannabinoids, there is variability regarding the activity of cannabinoid ligands at the receptor. For example, ∆9-THC and CP55,940 exhibit equal affinity for CB1 and CB2, whereas WIN55,212-2 exhibits modest selectivity for CB2 (2). Table 1 presents further detail regarding endogenous, naturally occurring and synthetic cannabinoids and their activity at receptors known to date.

Signal transduction at the CB1 receptor

Both cannabinoid receptor types are embedded in the cell membrane and are coupled to G proteins, negatively to adenyl
Cyclase and positively to mitogen-activated protein kinase (2, 6, 7). CB₁ receptors are coupled to ion channels through G proteins, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q-type calcium channels and to D-type potassium channels (2). Activation of either receptor will result in inhibition of adenylyl cyclase activity resulting in a decrease in the production of cyclic AMP (cAMP) and cellular activities dependent on cAMP, with opening of inwardly rectifying potassium channels resulting in decreased cell firing and closing of calcium channels resulting in decreased release of neurotransmitters (Figure 4). The overall effect is that of cellular inhibition. This is very much like the mechanism of action of the opioids. The cannabinoids and opioids have similar actions but involve different systems. The CB₁ receptor antagonist SR141716A prevents the analgesic effects of THC but not of morphine (34), whereas naloxone, an opioid antagonist, blocks the analgesic effect of morphine but not of THC and its analogues (35).

Thus, with regard to signal transduction at the CB₁ receptor, cannabinoids exhibit actions very much like the morphine group of drugs, but are able to act independently. The cannabinoid system is larger and occupies more areas than the opioid system, with the implication that the cannabinoid system may have wider potential therapeutic applications.

Endocannabinoid signalling in the brain
In contrast to classical neurotransmitters, investigators have identified that endocannabinoids are able to function as retrograde synaptic messengers (36). In this case, the endocannabinoid is synthesized and released from the postsynaptic neurons to travel backwards across the synapse, activating CB₁ on presynaptic axons and then suppressing neurotransmitter release. This capacity for ‘working backwards’ is directly relevant to pain modulation.

### Table 1
Cannabinoid agonists and antagonists*

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<thead>
<tr>
<th>Agent</th>
<th>Action</th>
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<tr>
<td>Naturally occurring cannabinoids</td>
<td>CB₁ and CB₂ agonist</td>
<td>Main psychoactive constituent of cannabis</td>
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<td>CB₁ receptors are coupled to ion channels through G proteins, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q-type calcium channels and to D-type potassium channels (2). Activation of either receptor will result in inhibition of adenylyl cyclase activity resulting in a decrease in the production of cyclic AMP (cAMP) and cellular activities dependent on cAMP, with opening of inwardly rectifying potassium channels resulting in decreased cell firing and closing of calcium channels resulting in decreased release of neurotransmitters (Figure 4). The overall effect is that of cellular inhibition. This is very much like the mechanism of action of the opioids. The cannabinoids and opioids have similar actions but involve different systems. The CB₁ receptor antagonist SR141716A prevents the analgesic effects of THC but not of morphine (34), whereas naloxone, an opioid antagonist, blocks the analgesic effect of morphine but not of THC and its analogues (35). Thus, with regard to signal transduction at the CB₁ receptor, cannabinoids exhibit actions very much like the morphine group of drugs, but are able to act independently. The cannabinoid system is larger and occupies more areas than the opioid system, with the implication that the cannabinoid system may have wider potential therapeutic applications.</td>
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Supraspinal sites of action

It has been demonstrated that cannabinoids act at multiple levels in the modulation of nociceptive or pain-related transmission (2,4,5). Intracerebroventricular administration of cannabinoids (37) suppresses tail-flick responses and spinal nociceptive responses (17). Direct brain injections into areas involved in descending inhibition of spinal nociceptive neurons elicits antinociceptive effects; these areas include the PAG in the midbrain, the RVM and the noradrenergic nucleus A5 in the medulla (38-40). Furthermore, microinfusion with the cannabinoid agonist WIN55,212-2 directly into the RVM in rats leads to increased ‘off-cell’ activity with increased tail-flick latencies, indicating that cannabinoids act directly within the RVM to affect off-cell activity (41). Additionally, cannabinoids have been shown to decrease noxious stimulus-evoked firing of nociceptive neurons in the ventral posterolateral nucleus of the thalamus as well as the RVM, with the latter being a demonstrated CB1 effect (4).

Through a series of experiments involving animal behaviour (tail flick) and extracellular single unit recordings from RVM neurons, along with administration of specific cannabinoid and opioid agonists and antagonists, it has been demonstrated that cannabinoids produce analgesia through the same brainstem circuit used for opioid analgesia. The use of an opioid is not required for the cannabinoid to produce this effect (42). In addition, both systemic and intracerebroventricular administration of cannabinoids have been shown to decrease noxious heat-evoked activity of wide dynamic range (WDR) neurons in a manner sensitive to spinalization, indicating a supraspinal site of action and descending modulation of WDR neurons (17).
PEA in the skin are enough to cause tonic activation of local cannabinoid receptors. Furthermore, the CB₁ antagonist SR141716A and the CB₂ antagonist SR144528 prolong and enhance pain behaviour following formalin injection. This work supports participation of endocannabinoids in the intrinsic control of pain initiation at peripheral sites (50).

CANNABINOIDS AND INTERACTIONS
WITH OTHER SYSTEMS

Monoaminergic/noradrenergic systems

There is evidence suggesting the involvement of monoaminergic systems in cannabinoid-induced antinociception. The serotoninergic neurotoxin 5,7-dihydroxytryptamine and the dopaminergic neurotoxin 6-hydroxydopamine both reduce the antinociceptive effect of cannabinoids in animal models. In these studies, noradrenergic involvement could not be ruled out due to the lack of pretreatment with a noradrenergic uptake inhibitor.

Intrathecal administration of yohimbine (an alpha-2-adrenergic antagonist) blocked antinociceptive effects of Δ⁹-THC. In contrast, intrathecal injection of the nonspecific neurotoxin 6-hydroxydopamine did not reduce Δ⁹-THC-induced antinociception, nor did serotonin depletion by p-chlorophenylalanine, suggesting a lack of serotonin involvement in cannabinoid antinociception. Similarly, the alpha₁-antagonist phenoxycyclamine failed to block cannabinoid antinociception. Taken together, these data support a role for the spinal noradrenergic system in cannabinoid-induced antinociception (3).

Opioid system

Studies have determined that the analgesic effect of THC is, at least in part, mediated through delta and kappa opioid receptors. THC administered intrathecally has been shown to release endogenous opioids that stimulate delta and kappa receptors (57). Delta antagonists do not interfere with cannabinoid antinociception. Dynorphin antisera and the selective kappa antagonist nor-binaltorphimine block THC-induced antinociception; this antagonism is specific to antinociception and occurs at the spinal level. Furthermore, dynorphin A (1-8) antiserum and antisense to the kappa-1 receptor antagonized the effect (2,3). In addition, a bidirectional cross tolerance of Δ⁹-THC and CP55,940 to kappa agonists has been demonstrated in the tail-flick test (58). Thus, the preponderance of data supports a role for kappa and delta opioid receptors in the mediation of a component of cannabinoid antinociception (57).

There is also some evidence supporting a possible role for mu opioid receptors in the enhancement of morphine antinociception by THC. Both naloxone and SR141716A (CB₁-specific antagonist) block the enhanced antinociception due to the combination of low-dose THC and morphine, supporting both CB₁ and mu opioid roles in the synergy (57). Thus, the current literature supports the possible involvement of all three major opioid receptor subtypes involved in some part in the enhancement of opioids by THC (57).

It has been demonstrated that cannabinoids can act synergistically with the opioid receptor agonists in the production of antinociception in animal models of acute pain (2,4). This synergy has been demonstrated in numerous studies, using several routes of administration (4), and the synergy works both ways, with cannabinoids enhancing opioid antinociception and morphine enhancing cannabinoid antinociception. Full isobolographic analysis has substantiated the greater than additive effect necessary to identify synergy (57).

Following chronic dosing, upregulation of opioid receptor protein in the spinal cord has been observed in combination-treated animals and may play a role in retention of efficacy of the drug combination. Short-term administration of low-dose THC with morphine in mice attenuated opioid tolerance without the loss of the antinociceptive effect. Further prolonged exposure to a cannabinoid agonist failed to result in downregulation of delta opioid receptors in vitro. Taken together, these results support that cannabinoids can alter opioid tolerance (57). Thus, data support a synergistic effect of cannabinoids and opioids and a possible role for cannabinoids in situations of opioid tolerance.

CANNABINOIDS AND PAIN

Cannabinoids exhibit antinociceptive and antihyperalgesic effects in models of acute and chronic pain

Preclinical work reveals that cannabinoids block pain responses in virtually every pain model tested. One of the earliest studies was performed by Dixon (59), who demonstrated that cannabis was able to suppress canine reactions to pinpricks. In models of acute or physiological pain, cannabinoids are effective against thermal, mechanical and chemical pain and are comparable with opioids in potency and efficacy (5).

In models of chronic pain, cannabinoids exhibit greater potency and efficacy in both inflammatory (60) and neuropathic pain (61). Because cannabinoids are also able to affect motor systems, it is important to establish that the slowed reactions of animals in pain tests are not because of slowed motor activity rather than pain inhibition. In electrophysiological studies, it has been concluded that cannabinoids produce profound suppression of cellular nociceptive responses with no suppression of the low threshold mechanoreceptive neurons (5). These experiments include suppression of neurophysiological responses to all types of nociceptive stimuli tested, suppression of windup (a model of central sensitization observed in chronic pain) and suppression of increased spontaneous firing following injection of the inflammatory agent complete Freund’s adjuvant (2,5,17,18,62-65). Thus, there is significant evidence that cannabinoids exhibit antinociceptive and antihyperalgesic effects in models of acute and chronic pain.

Of further importance to chronic pain is the fact that upregulation of CB₁ receptors (within the ipsilateral superficial dorsal horn of the spinal cord in rats following chronic constriction injury of the sciatic nerve) has been demonstrated. This enhanced the effects of a cannabinoid agonist (WIN55,212-2) on both thermal hyperalgesia and mechanical allodynia, supporting that upregulation of spinal cannabinoid receptors following peripheral nerve injury may contribute to the effects of exogenous cannabinoids in neuropathic pain (66). Furthermore, repeated administration of WIN55,212-2 given subcutaneously reversed the development of hyperalgesia that normally develops in chronic constriction of the sciatic nerve in rats (67), supporting that cannabinoids may play a role in prevention of neuropathic pain if given early after nerve injury.

Nonpsychoactive cannabinoids targeting pain

There is significant interest in the development of synthetic cannabinoids without psychotropic activity (68-70). Ajulemic acid (also called CT-3) is a synthetic analogue of Δ⁹-THC-11-oic-acid, one of the endogenous transformation products of THC.
In preclinical studies, ajulemic acid has been found to exhibit potent analgesic, antiallodynic and anti-inflammatory activity; however, it binds to CB3 receptors and has been found to cause sedation in mice (70). Cannabidiol (CBD) is a nonpsychoactive cannabinoid present in cannabis that does not bind to cannabinoid receptors; it has also been demonstrated that CBD inhibits FAAH and blocks the reuptake of AEA, thus enhancing extracellular levels of AEA (71). Investigators have developed synthetic analogues to CBD in a search for a nonpsychoactive, nonsedating agent. HU-320 (CBD-dimethyl-heptyl-7-oxo acid) is a novel synthetic cannabinoid acid that has been demonstrated to exhibit strong anti-inflammatory and immuno-suppressive properties while demonstrating no psychoactive effects (70).

**Anti-inflammatory and peripheral antihyperalgesic effects of cannabinoids**

Following tissue injury or inflammation with disruption in normal tissue integrity and migration of various cells (eg, immune and mast cells, platelets), a diversity of chemical mediators are produced or released locally. These mediators then activate peripheral sensory nerve endings. Some will activate the sensory nerve directly; others will sensitize the nerve to other stimuli or exert regulatory effects on the sensory neuron, inflammatory cells and adjacent sympathetic nerves (Figure 5) (72).

There is evidence that CB2 and CB3 receptors are present peripherally, and the mechanisms for synthesizing, releasing and inactivating endocannabinoids are present during inflammation (7).

CB3 agonists exhibit a direct effect on the sensory nerve terminal itself to inhibit release of calcitonin gene-related peptide (51) and inhibit sensitizing effects of nerve growth factor (NGF) (7). Peripheral administration of AEA attenuates hyperalgesia and edema via a CB3 receptor mechanism and inhibits capsaicin-evoked plasma extravasation into the hindpaw (51).

Local analgesic actions of directly and indirectly acting agonists for CB3 receptors, expressed on mast cells and inhibiting mast cell function, have also been demonstrated (50,52). CB3 receptor mechanisms may play a particularly prominent role in inflammatory pain (7). Both CB3 and high-affinity NGF receptors (trkA) have been identified on mast cells, and mast cells amplify the NGF signal during inflammation (7). There is increasing evidence that PEA (a CB3 agonist) attenuates this amplification. PEA accumulates in inflamed tissue, is synthesized by leukocytes, prevents mast cell degranulation and suppresses inflammatory hyperalgesia and edema (7). Furthermore, it has been demonstrated that neutrophil migration is diminished by endocannabinoids in models of inflammatory pain. In addition, cannabinoids attenuate nitric oxide production from stimulated macrophages via a CB3 receptor-mediated action (7), and have also been demonstrated to have profound and complex effects on cytokine production (73).

A CB3 selective agonist (AM1241, administered intraperitoneally) suppressed development of intradermal capsaicin-induced thermal and mechanical hyperalgesia and allodynia; this was reversed by a CB3 antagonist (SR144528) but not by a CB3 antagonist (SR14716A). Also, AM1241 suppressed thermally and mechanically evoked hyperalgesia and allodynia following local administration to the capsaicin ipsilateral paw but had no effect on the contralateral (untreated) paw. These data provide evidence that actions at CB3 receptors are sufficient to normalize nociceptive thresholds and produce antinociception in persistent pain states (74).

In animal models of inflammatory pain, local administration of AEA, PEA and synthetic cannabinoids have been repeatedly demonstrated to attenuate behavioural responses to proinflammatory substances including subcutaneous formalin, capsaicin and complete Freund’s adjuvant (7). A recent study (75) found that nabilone, a cannabinoid agonist available by prescription in Canada, reduced edema and associated hyperalgesia following carrageenan injection into the paw. It has also been demonstrated that AEA causes inhibition of interleukin-2 secretion in activated splenocytes via a mechanism involving both cyclooxygenase-1 and cyclooxygenase-2 (76). Old anti-inflammatory analgesic drugs such as indomethacin and flurbiprofen activate CB3 receptors via a decrease in FAAH degradation and, therefore, an increase in AEA concentration, suggesting the potential for a cannabinoid mechanism of action contributing to their effects (77).

**Visceral pain conditions**

Manipulation of CB3 receptors can alter sensory processing from the gut; brain integration of the brain-gut axis; extrinsic control of the gut; and intrinsic control by the enteric nervous system (78).

The upper gastrointestinal tract is strongly influenced by CB3 receptor activation on central vagal pathways, whereas intestinal peristalsis can be modified by CB3 receptor activation in the absence of extrinsic input (78). Endocannabinoids (AEA and PEA) attenuate viscer-visceral hyperreflexia, spinal Fos expression and the referred hyperalgesia in a model of cystitis that shares features of interstitial cystitis; the effects...
of AEA are predominantly CB₁ receptor-mediated and the effects of PEA are predominantly CB₂ receptor-mediated (7). CB₁-deficient mice or wild-type mice administered CB₁ antagonists exhibit increased inflammation following intrarectal administration of proinflammatory substances (e.g., 2,4-dinitrobenzene sulphonic acid [DNBS]). Treatment with a cannabinoid agonist or genetic ablation of FAAH protected against the development of DNBS-induced colitis. Electrophysiological recordings from circular smooth muscle cells 8 h after the administration of DNBS revealed spontaneous action potentials in CB₁-deficient mice but not in wild-type littermate colons, indicating early CB₁-mediated control of inflammation-induced irritation of smooth muscle cells. DNBS treatment increased the percentage of myenteric neurons expressing CB₁ receptors, suggesting enhanced cannabinoid signalling during colitis. This work supports evidence that CB₂ receptors mediate intrinsic protective signals that counteract proinflammatory responses and indicates the endocannabinoid system is a promising target for the treatment of gastrointestinal disorders with excessive inflammatory responses (79).

The future of cannabinoid research

The present review has focused on cannabinoid research relating to pain applications. As presented in the introduction, there are many other potential applications for cannabinoid agonist and antagonist molecules under development. Perhaps the most exciting area of research regarding cannabinoids is the identification of ways to manipulate the endocannabinoid system. Unlike endogenous opioids, endocannabinoids are synthesized by what appear to be relatively selective enzymes. Furthermore, there is also intense focus on the mechanism of reuptake and inactivation of the endocannabinoids. In the future, it may be possible to manipulate the endocannabinoid system for the treatment of pain in much the same way as the monoaminergic system is targeted for the treatment of depression.

CONCLUSION

The potent antinociceptive and antihypalgesic effects of cannabinoid agonists, the presence of cannabinoid receptors in pain-processing areas of the brain, spinal cord and periphery, and the endogenous modulation of pain systems by cannabinoid support that cannabinoids exhibit significant potential as analgesics.

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