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An overview of the cannabinoid type 2 (CB2) receptor system and its therapeutic potential

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Abstract

Purpose of review—This narrative review summarizes recent insights into the role of the CB2 receptor as potential therapeutic target in neuropathic pain and neurodegenerative conditions.

Recent findings—The cannabinoid system continues to receive attention as a therapeutic target. The cannabinoid type 2 (CB2) receptor is primarily expressed only when there is active inflammation and appears to be devoid of undesired psychotropic effects or addiction liability. The CB2 receptor has been shown to have potential as a therapeutic target in models of diseases with limited or no currently approved therapies, such as neuropathic pain and neurodegenerative conditions such as Alzheimer's disease.

Summary—The functional involvement of CB2 receptor in neuropathic pain and other neuroinflammatory diseases highlights the potential therapeutic role of drugs acting at the CB2 receptor.

Keywords

Cannabinoid receptor type 2 (CB2); microglia; neuroinflammation

Introduction

Cannabinoid receptors, located throughout the body, are part of the endocannabinoid system, which is involved in a variety of physiological processes. The cannabinoid system continues to receive attention as a therapeutic target. Broad claims for marijuana and its derivatives are being made but these remain to be proven in carefully controlled trials and they are burdened by the side effect profile of the cannabinoid type 1 (CB1) receptor.

The cannabinoid type 2 (CB2) receptor is primarily expressed only when there is active inflammation and appears to be devoid of undesired psychotropic effects or addiction

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Conflicts of interest

MN and JFF are cofounders of *NeuroTherapia*™, a spin-off company created by Cleveland Clinic Innovations. MN and JFF have not received payments from *NeuroTherapia*™. As inventors of the technology, they and Cleveland Clinic are entitled to future royalty payments. JW and BB declare no competing interests.

liability. The CB2 receptor has been shown to have potential as a therapeutic target in models of diseases, such as neuropathic pain and neurodegenerative conditions such as Alzheimer's disease, where activation of the microglia and neuroinflammation are present. Significant advances have been made in the understanding of the CB2 receptor system, its role in controlling neuroinflammation, and potential therapeutic actions. These findings need to be taken into account in the discovery and evaluation of potential new drugs.

The endocannabinoid system

The endogenous cannabinoid system encompasses two cannabinoid (CB) receptors, endogenous ligands, and several enzymes required for biosynthesis and inactivation of endogenous ligands [1] (Figure 1). CB1 receptors are expressed primarily in the brain [2] and to some extent in the peripheral tissues.[3–5] CB2 receptors are identified peripherally in the circulating immune cells, the spleen [6, 7], and on macrophage-derived cells including osteocytes, osteoclasts, and hepatic Kupffer cells.[8, 9] Unlike the widespread expression of CB1 in the CNS, the expression of CB2 receptors, under normal physiological conditions, is restricted to the brainstem and the hippocampal CA2/3 pyramidal neurons.[10, 11] However, CB2 expression is highly inducible on the reactive microglia in the CNS following inflammation or injury.[12–20] Both CB1and CB2 receptors are seven transmembrane, G-protein-coupled receptors, and they share 44% overall identity. Studies performed with $CB1^{-/-}$ and $CB2^{-/-}$ mice have indicated that certain effects of cannabinoids on tissues are mediated by neither CB1 nor CB2 [21], but currently no additional cannabinoid receptors have been definitively identified.[22] The crystal structures of CB1 (but not CB2) receptor in both active and inactive states have been reported.[23, 24]

The endocannabinoid system also includes two arachidonic acid derivatives ligands—anandamide and 2-arachidonoylglycerol (2-AG) [1], two enzymes responsible for synthesizing endogenous ligands—1,2-diacylglycerol lipase and phospholipase A, and two enzymes responsible for the metabolism of endogenous ligands—fatty acid amide hydrolase and monoglyceride lipase.[25] The endocannabinoid 2-AG was found to act as a full agonist (whereas anandamide acts as a weak partial agonist) toward CB1 and CB2 receptors.[26] In the CNS, the release of *postsynaptic* endocannabinoid ligands activate the *presynaptic* CB1 receptors in a retrograde manner leading to inhibition of calcium channels and activation of potassium channels, resulting in inhibition of presynaptic neurotransmission release.[27–29]

The naturally-occurring cannabis family encompasses three major species (*Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis)* and the cannabis plant contains more than 500 natural compounds that include more than 100 cannabinoids.[30] The full characterization of all cannabinoids is still lacking. Marijuana is composed mainly of the dried buds of *Cannabis sativa*. Delta-9-tetrahydrocannabinol (Δ^9-THC) is the primary psychoactive cannabinoid of marijuana [31, 32] that acts as an agonist on the CB1 receptor [33] and as a weak antagonist on the CB2 receptor.[34] Cannabidiol is another major component of the cannabis that acts a non-competitive negative allosteric modulator of CB1 receptors.[35, 36] The claims that marijuana has efficacy in different medical conditions have not been substantiated.[37]

CB2 and microglia in neuroinflammatory conditions

Glial cells—including parenchymal (resident) microglia, perivascular microglia, astrocytes, and oligodendrocytes—constitute more than 70% of the total cell population in the brain and spinal cord and represent the first line of defense against inflammation and other insults.[38, 39] Microglia are the resident immune cells of the brain and play a vital role in health and disease.[16] Under normal physiological conditions (Figure 2a), microglia play a central role in the induction and maintenance of synaptic plasticity in neurons by altering the local environment and by modifying synaptic structure.[40–45] Phagocytosis of synapses by microglia contributes to synaptic development [46] and synaptic deficit in neurological disorders.[47–50] Microglial activation occurs in response to diverse CNS insults, and as a result, a transition is seen in microglial phenotype [51] from healthy anti-inflammatory to the reactive (proinflammatory) phenotype (Figure 2b). The reactive microglia express several receptors such as Toll-like receptors (TLRs)[17] and purinergic P2X4 receptors [52] with the subsequent activation of inflammatory pathway and release of several inflammatory cytokines and chemokines that eventually result in neuronal damage. Microglial activation and neuroinflammation appear to be the upstream mechanism underlying the pathogenesis of neurodegenerative diseases—including neuropathic pain, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, AIDS, and Huntington's disease.[12, 13, 16–19, 53]

Reactive microglia express CB2 mRNA in the spinal cord under neuropathic pain conditions.[54] The expression of CB2 receptors (Figure 2b) is increased in the dorsal horn in different models of neuropathic pain such as peripheral nerve injury, chemotherapy-induced neuropathic pain, and chronic post-ischemia pain (a model of complex regional pain syndrome type 1), and this expression is colocalized with the activated microglia.[17, 20, 54–57]

CB2 receptors were identified in postmortem brain tissues of patients with Alzheimer's disease [14, 58, 59] and CB2 receptors were substantially and selectively expressed in neuritic plaque-associated microglia.[12] CB2 receptors are also upregulated in reactive microglial cells in animal models of Alzheimer's disease, Huntington's disease, simian immunodeficiency virus–induced encephalitis, HIV encephalitis, and multiple sclerosis.[13, 17–19, 60]

CB2 agonists modulate central neuroinflammatory conditions

In many physiologic stress settings (e.g., wound healing), a negative-feedback loop helps to reestablish homeostasis. In some inflammatory settings, activation of toll-like receptors (TLRs) in the microglia induces the release of cytokine signaling protein suppressors,[61] and tumor necrosis factor alpha (TNFα)-induced protein 8 family members [62] tend to limit inflammatory responses via modulation of TLRs. The upregulation and activation of the CB2 receptor may be part of the active process of limiting or downregulating the inflammatory process (much like tumor suppressors in cell growth or ephrin receptors in the nervous system, which can have positive or negative effects, depending on the context).[63] Activation of the CB2 receptor has been shown by different investigators to limit the acute inflammatory process.[17, 19, 20, 33, 64–69]

CB2 agonists are neuroprotective and lack psychotropic adverse effects normally seen with CB1 agonists. Activation of the CB2 receptor system results in inhibition of neuroinflammatory signaling pathways, restoration of normal microglial function (from pro-inflammatory to anti-inflammatory state). CB2 receptors modulate the extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) activation.[70] Both TLR2 and TLR4 are linked to the phosphorylation of ERK1/2.[17, 71] In vitro evidence indicates that the endocannabinoid anandamide acts through the mitogen-activated protein kinase (MAPK) pathway within the CNS immune system to reduce the extent of the inflammatory response and to limit neurodegenerative immune reactions.[70] We have shown that using a specific CB2 agonist, 1-(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl)carbonyl) piperidine (MDA7)[72] modulated the expression of the genes in paclitaxel-induced neuroinflammatory response as evidenced by relatively reduced expression of TLR2 and CB2 receptors and ERK1/2 expression (Figure 3).[73] In addition, the use of MDA7 was also associated with the adaptation of spinal glutamatergic transmission.[73]

In neuropathic pain models, treatment with CB2 agonists resulted in prevention of mechanical allodynia in different animal models (spinal nerve ligation model, paclitaxel-induced neuropathy, and chronic post-ischemic pain model of complex regional pain syndrome type I), and in animal models of Alzheimer's disease, treatment with CB2 agonists promoted the clearance of amyloid plaques and recovery of the neuronal synaptic plasticity (Figure 2c).[17–20, 73–85]

In neuropathic pain conditions, glial activation and neuroinflammation are not restricted to the dorsal horn but are seen in several brain regions that functionally regulate the pain perception and sensitivity.[86] For example, sciatic nerve ligation induces upregulation of immature metabotropic glutamate receptor 5 and spontaneous somatic Ca^{2+} transients in astrocyte in the S1 sensory cortex, which contributes to the enhanced synaptic plasticity in S1 sensory cortex and mechanical allodynia in rodents.[87] Increased expression of TLR4 in glia and production of proinflammatory cytokines were noted in the prefrontal cortex in mice under chronic stress, and knockout of TLR4 or pharmacological suppression of TLR4 reduced visceral pain and prevented the development of chronic psychosocial stress-induced visceral hypersensitivity.[88] Significant activation of microglia and astrocytes was also observed in the anterior cingulate cortex (ACC) of the mice with nerve-ligation, and intracerebroventricular or/and intra-ACC injection of minocycline suppressed the phosphorylation of GluR1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor at Ser^{831} and mechanical hypersensitivity in the modeled rodents.[89] A recent clinical study using integrated positron emission tomography-magnetic resonance imaging showed increased brain concentrations of the translocator protein (TSPO), a marker of glial activation, in patients with chronic low back pain.[90]

CB2 agonists modify opioid-induced tolerance and reward seeking behavior

Microglial activation seems to be involved in opioid-induced tolerance and addiction. Chronic opioid administration induces microglial activation and the release of pro-inflammatory cytokines and chemokines.[91, 92] Inhibiting microglial activation mitigated the development of tolerance, opioid-induced reward mechanism [93, 94], and restored the

analgesic efficacy of opioids.[95] Systemic administration of a CB2 agonist (JWH133) attenuated both the rewarding and the psychomotor-stimulating effects of cocaine.[96]

CB2 agonists modulate peripheral neuroinflammation

Local injection of complete Freund's adjuvant into the hindpaw of rodents increased the mRNA and protein concentrations of CB2 receptors in the skin tissue, which were primarily distributed in keratinocytes, macrophages, and T-lymphocytes in the epidermis and dermis of the inflamed skin tissue.[97] Stimulation with lipopolysaccharide enhanced the expression of CB2 receptors and the production of pro- and anti-inflammatory factors in human keratinocytes and fibroblasts. Administration of a CB2 receptor agonist JWH015 [98] or AM1241 [99, 100] reduced the concentration of major pro-inflammatory factors and increased the concentration of a major anti-inflammatory factor (TGF-β) in lipopolysaccharide-stimulated human keratinocytes and fibroblasts cells or inflamed skin tissues.

Therapeutic potential of CB2 agonists

Several CB2 agonists are described in the literature and a patent review indicated that there are several CB2 receptor modulators are in different phases of clinical development.[101] An effective molecule should be able to cross the blood brain barrier to reach the CNS to act on reactive microglia and it should be tested in the proper patient population. In this context, it is always insightful to study the earlier failures of CB2 agonists.

GlaxoSmithKline's CB2 agonist, GW842166X, which appears to have a limited CNS permeability, was reported to be effective in the Freund's complete adjuvant model of inflammatory pain (with an oral ED_{50} of 0.1 mg/kg).[102] CB2 ligands are known to have limited or no efficacy in acute pain models.[74, 103] CB2 agonists are not effective in treating acute pain in rats [74] and administration of the CB2 receptor–selective agonist HU-308 did not affect acute nociception in mice when thermal withdrawal latency was measured on a hot plate.[103] Administration of GW405833 did not affect hot plate or tail flick latency after administration of doses up to 30 mg/kg. However, 100 mg/kg of GW405833 resulted in a significant increase in both tail flick and hot plate latencies 1 h after administration.[104] The 100 mg/kg dose of GW405833 also resulted in typical CB1 effects [104], which does not support a CB2-mediated effect on acute nociception. GW842166X was subsequently found to be inferior to ibuprofen in a phase II randomized-controlled trial in patients with acute pain following third molar tooth extraction.[105] The authors discuss the possibility that plasma levels were suboptimal, although the plasma levels obtained were greater than those in preclinical studies demonstrating efficacy in the rat model.

AstraZeneca CB2 ligand, AZD1940, is a peripherally restricted CB1/CB2 receptor agonist. [106] AZD1940 has a low brain uptake at analgesic doses in both rats and primates.[107] Dose-dependent CNS-related and gastrointestinal adverse events were reported following treatment with AZD1940 in healthy male volunteers.[106] These effects were induced by the activity of AZD1940 at CB1 receptors. AZD1940 was not effective in the human capsaicin pain model[106] and did not reduce postoperative pain after lower third molar surgical removal at doses exerting subjective cannabinoid effects.[108] As stated by Rogers:

"Choosing a human study group for convenience, which is kind of how osteoarthritis and third molar extraction got chosen, isn't necessarily matching with what the preclinical results suggest may be a good patient population to study".[109]

Conclusion

The CB2 receptor is primarily expressed only when there is active inflammation and appears to be devoid of undesired psychotropic effects or addiction liability. The CB2 receptor has been shown to have potential as a therapeutic target in models of diseases with limited or no currently approved therapies, such as neuropathic pain and neurodegenerative conditions such as Alzheimer's disease. The challenge ahead is to identify drug candidates selective for CB2 which reaches the receptors in the CNS, and to subsequently demonstrate efficacy in clinical trials that represent the known mechanism of action of the CB2 system.

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Key Points

- CB2 expression is highly inducible on the reactive microglia in the CNS following inflammation or injury.
- Activation of CB2 receptor suppressed reactive microglia behavior and central neuroinflammation, and demonstrated a protective role in neuroinflammatory conditions.
- Preclinical studies showed that CB2 agonists might modify opioid-induced tolerance and reward seeking behavior.
- Several CB2 receptor modulators are in different phases of clinical development targeting chronic pain treatment.

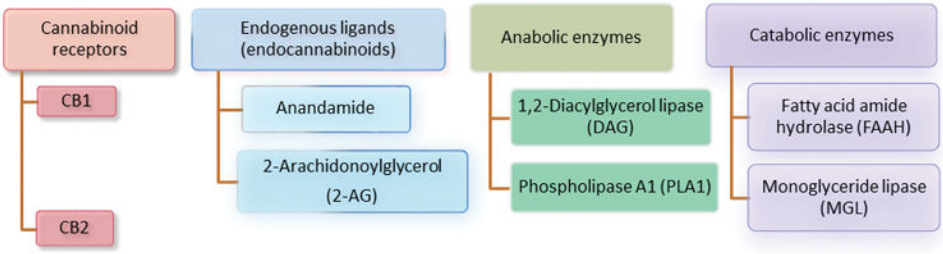

Figure 1.

Components of the endogenous cannabinoid system. The cannabinoid (CB) receptors CB1 and CB2 belong to the G-protein-coupled receptor superfamily, coupled to Gi/o proteins and, under certain conditions, coupled to Gs. CB1 receptors are expressed mainly in the brain and CB2 are expressed mostly in the peripheral immune system and in the CNS in the hippocampal CA2/3 pyramidal neurons and glial cells. The endocannabinoid system also includes two arachidonic acid derivatives ligands (anandamide and 2-arachidonoylglycerol) [1], two enzymes responsible for synthesizing endogenous ligands (1,2-diacylglcerol lipase and phospholipase A), and two enzymes responsible for the metabolism of endogenous ligands (fatty acid amide hydrolase and monoglyceride lipase).

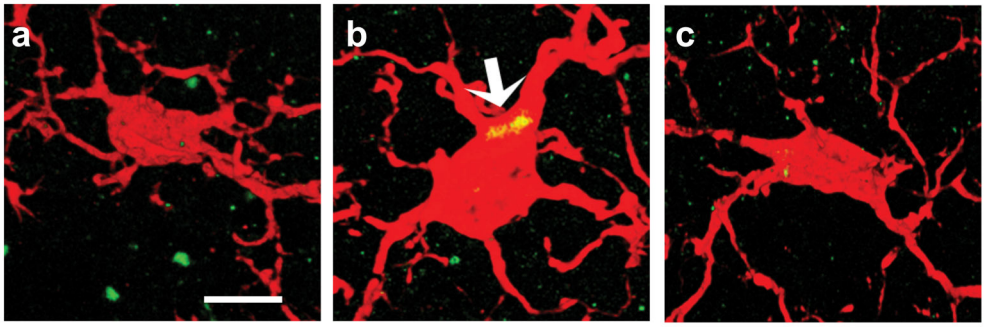

Figure 2.

Cannabinoid type 2 (CB2) receptors are expressed in reactive microglia in different neuroinflammatory and neurodegenerative conditions such as neuropathic pain and Alzheimer's disease. The figure depicts 3D immunofluorescence confocal images of the microglial marker ionized calcium binding adaptor molecule (1 Iba1) (red) and the immunosignal of cannabinoid type 2 (CB2) receptor (green) in microglia; the colocalization of CB2 and microglia is shown in yellow. No substantial CB2 expression is seen in the healthy microglia (**a**), but increased expression of CB2 is seen in reactive microglia (arrow, **b**). Note the change from a highly branched and ramified morphology under normal physiological conditions to an amoeboid form in the presence of neuroinflammation. Treatment with a selective CB2 agonist, 1-(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl)carbonyl) piperidine (MDA7) restored microglial function and normal morphology (**c**). Scale bar = 10 μm.

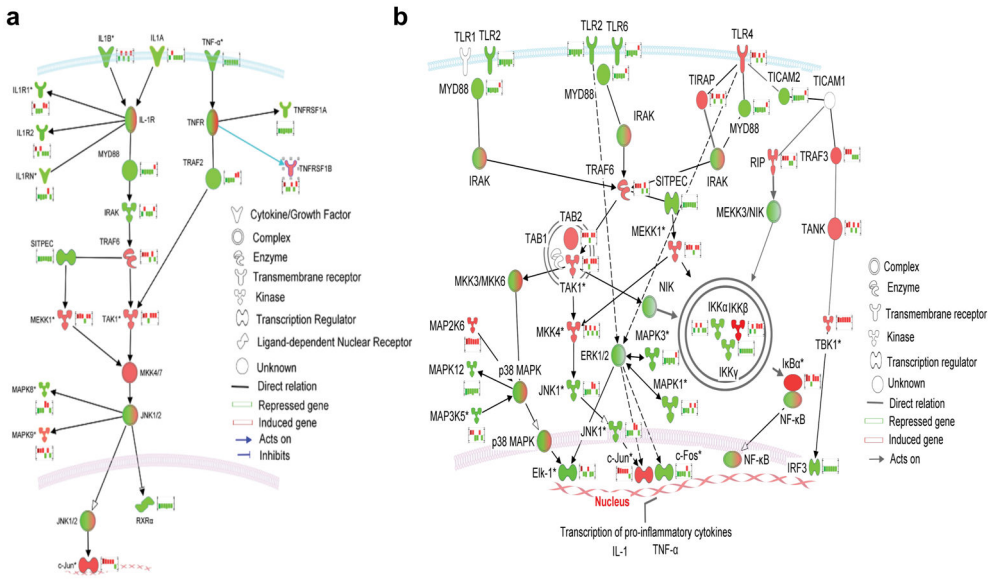

Figure 3.

(a) Modulation of interleukein-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) pathways by a selective CB2 agonist, 1-(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl)carbonyl) piperidine (MDA7) (generated by Ingenuity pathway analysis using comparison analysis between the 6 arrays). The brightness of node colors is proportional to the fold changes of gene expression levels. Color indicates up-regulated (red) and down-regulated (green) genes. In addition fold change expression bar charts of the six arrays have been included for each gene. In the IL-1 pathway, a complex is formed including IL-1R-associated kinases (IRAK) and the adapter protein MyD88. IRAK is rapidly phosphorylated and associates with TNF receptor-associated factor 6 (TRAF6); this association is necessary for downstream IL-1-induced translocation of signaling molecules to the nucleus, which ultimately leads to expression of genes that mediate inflammation and, frequently, tissue destruction. MDA7 acts by inhibiting IL-1 and TNF-α decreased expression of MyD88 and TNF receptor-associated factor 2 (TRAF2). **(b)** Signaling cascades initiated *via* toll-like receptor 2 (TLR2)- and TLR4-dependent activation and its modulation by MDA7. In addition fold change expression bar charts of the six arrays have been included for each gene. Engagement of TLR2 on the cell surface as a heterodimer with either TLR1 or TLR6 leads to recruitment of the adaptor protein MyD88 and interaction with TIR-domain-containing adaptor protein (TIRAP) via death-domain interactions. Phosphorylated IL-1 receptor-associated kinase (IRAK), together with TNF receptor-associated factor 6 (TRAF6), dissociates from the receptor, and then TRAF6 interacts with TAK1 binding protein 1 (TAB1)—induces autophosphorylation of the transforming growth factor β (TGFβ)-activating kinase (TAK1), and TAB2. This leads to phosphorylation of the IκBα-kinase (IKK) complex (IKKα, IKKβ, and IKKγ) and mitogen-activated protein kinases (MAPK), such as c-Jun NH2-terminal kinase (JNK), inducing the nuclear translocation of NF-κB and subsequent induction of target genes such as TNFα and ILs. The transcriptional activity of NF-κB is tightly regulated by its association with the inhibitory IκB that sequesters NF-κB in the cytosol. Beneficial effects of MDA7 include modulation of general genes that will ultimately inhibit phosphorylation of IκB proteins by the IKKs and prevention of IκB

degradation. TLR4-MyD88-independent pathway activation involves signaling through the Toll-interleukin-1 receptor (TIR) adaptor TRIF (also known as TICAM1), TRIF-related adaptor molecule (TRAM; also known as TICAM2), TRAF3, receptor-interacting protein (RIP) and the transcription factor interferon regulatory factor 3 (IRF3). The green and red bar charts and shading represent the respective differential gene modulation (repression or induction, respectively) by MDA7. The numbers represent fold change. (Reproduced from Xu et al.[73] with permission).