

Review

Brain cannabinoid receptor 2: expression, function and modulation

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Abstract

Cannabis sativa (marijuana) is a fibrous flowering plant that produces an abundant variety of molecules, some with psychoactive effects. At least 4% of the world's adult population uses cannabis annually, making it one of the most frequently used illicit drugs in the world. The psychoactive effects of cannabis are mediated primarily through cannabinoid receptor (CBR) subtypes. The prevailing view is that CB₁Rs are mainly expressed in the central neurons, whereas CB₂Rs are predominantly expressed in peripheral immune cells. However, this traditional view has been challenged by emerging strong evidence that shows CB₂Rs are moderately expressed and function in specific brain areas. New evidence has demonstrated that brain CB₂Rs modulate animal drug-seeking behaviors, suggesting that these receptors may exist in brain regions that regulate drug addiction. Recently, we further confirmed that functional CB₂Rs are expressed in mouse ventral tegmental area (VTA) dopamine (DA) neurons and that the activation of VTA CB₂Rs reduces neuronal excitability and cocaine-seeking behavior. In addition, CB₂R-mediated modulation of hippocampal CA3 neuronal excitability and network synchronization has been reported. Here, we briefly summarize recent lines of evidence showing how CB₂Rs modulate function and pathophysiology in the CNS.

Keywords: cannabis; brain cannabinoid receptor 2; GPCR; neuronal excitability; psychiatric disorders; neurological disorders; drug addiction

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Introduction

The cannabinoid type 2 receptor (CB₂R) is a G-protein-coupled receptor that was cloned in 1993^[1]. Since then, the expression and function of CB₂Rs in the brain have been debated. Early studies suggested that CB₂Rs were absent in the brain because CB₂ mRNA transcripts were not detected in brain tissue using various methods^[2–5]. Based on these findings, CB₂Rs have been considered the “peripheral” cannabinoid receptor^[1, 6, 7]. Recently, this concept has been challenged by the identification of CB₂Rs throughout the central nervous system (CNS)^[5, 6]. Compared with CB₁Rs, brain CB₂Rs exhibit several unique features: (1) Brain CB₂Rs have lower expression levels than CB₁Rs, suggesting that CB₂Rs may not mediate the effect of cannabis under normal physiological conditions. (2) Brain CB₂Rs are highly inducible; thus, under some pathological conditions (eg, addiction, inflammation, anxiety), CB₂R expres-

sion is quickly enhanced in the brain^[8]. This finding suggests a close relationship between the alteration of CB₂R expression/function and various psychiatric and neurological diseases. (3) Brain CB₂Rs have a specific distribution. Given that they are chiefly expressed in neuronal somatodendritic areas^[9] (post-synaptic), the activation of CB₂Rs may lead to opposing effects from CB₁Rs. For example, CB₁Rs are predominantly expressed on neuronal terminals, especially on GABAergic terminals (presynaptic) in ventral tegmental area (VTA) dopamine (DA) neurons^[10]. The activation of CB₁Rs reduces GABA release onto DA neurons, leading to an increase in DA neuronal firing through a disinhibition mechanism. However, CB₂Rs are mainly located on postsynaptic somatodendritic areas, and the activation of CB₂Rs reduces VTA DA neuron firing and excitability^[11]. Considering these characteristics, CB₂Rs appear to be an important substrate for neuroprotection^[12], and targeting CB₂Rs will likely offer a novel therapeutic strategy for treating neuropsychiatric and neurological diseases without typical CB₁R-mediated side effects^[13]. Thus, an urgent need to understand the functional effects of CB₂Rs in the brain has emerged.

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Unfortunately, CB₂R-mediated modulation of neuronal functions, including ion channels, receptors, synaptic transmission and plasticity, and neuronal networks in the CNS, has not been well investigated, and to date, studies of the functional effects of CB₂R in neurons have ignited debate and controversy due to the following reasons: (1) a lack of highly selective CB₂R antibodies^[14]; (2) a lack of full knockout (KO) CB₂R mice as the two types of CB₂R KO mice that have been recently made available are partial knockouts^[7]; and (3) under some conditions, CB₁R and CB₂R can form a heteromer^[15], which makes the identification of CB₂R function even more complex and difficult. Nevertheless, by combining multiple experimental approaches, several recent papers have described CB₂R expression and function in the brain neurons. Recently, we reported that functional CB₂R was expressed in VTA DA neurons, and activation of VTA CB₂R reduces neuronal excitability and cocaine-seeking behavior^[11]. It has been reported that CB₂R modulates hippocampal CA1 synaptic plasticity^[16] and CA3 neural plasticity and synchronization^[17]. In addition, a recent report showed that CB₂R-dependent inhibition of DA release underlies the positive allosteric modulation of the M4 muscarinic receptor in antipsychotic-like effects^[18]. These accumulating lines of evidence suggest that brain CB₂R is important in the modulation of brain function and disorders.

Brain CB₂R expression

Although early studies were not able to detect CB₂ mRNA transcripts in brain tissue using various methods^[2-5], recent lines of evidence show that significant CB₂ mRNA has been detected by *in situ* hybridization (ISH) in the globus pallidus of non-human primates^[19]. RT-PCR analysis has also been used to detect CB₂ mRNA expression in various brain regions, including the retina^[20], cortex^[19, 21-23], striatum^[2, 23], hippocampus^[17, 19, 24], amygdala^[22, 23], brainstem^[25], and cerebellum^[26]. Furthermore, two CB₂ mRNA transcripts (CB_{2A} and CB_{2B}) are transcribed from two independent promoters in rodent and human tissue^[21]. Recently, we have confirmed that CB₂ mRNA is relatively highly expressed in VTA DA neurons^[11]. Moreover, immunoblot and immunohistochemical (IHC) assays have detected significant CB₂-like bands or immunostaining in various brain regions^[25, 27-30], including the VTA^[11]. These findings suggest that CB₂R expression exists not only in peripheral tissue but also in the brain, although the CB₂R expression level in the brain is much lower than that of CB₁R. This low-level brain CB₂R expression suggests that CB₂R may not participate in important brain physiology function; thus, unlike CB₁R that mediate serious psychiatric side effects after activation, pharmacological intervention of CB₂R has much fewer side effects. In addition to brain neurons, brain glia cells express CB₂R^[31, 32], where CB₂R play an important role in the modulation of central immune function^[33] and neuroinflammation-associated diseases^[34-36].

Brain CB₂R distribution and function

Although both CB₁R and CB₂R are G-protein (G_{i/o})-coupled receptors, they exhibit different distributions. In general, the

CB₁R are mainly expressed in GABAergic neuronal axon terminals, including in VTA GABA neurons^[37, 38] and hippocampal CCK-positive GABA neurons^[39]. The activation of CB₁R reduces presynaptic GABA release, eliminates GABAergic inhibitory control of postsynaptic neurons, and excites these postsynaptic neurons through this dis-inhibition role. However, the CB₂R are mainly expressed in the postsynaptic cell body^[11, 17]; thus, the activation of these postsynaptic CB₂R usually hyperpolarizes the membrane potential and inhibits postsynaptic neuronal function. Therefore, this difference in distribution results in opposite effects after CB₁R and CB₂R activation. Activation of CB₂R reduces neuronal excitability through different mechanisms. In VTA DA neurons, activation of CB₂R decreases neuronal excitability through the CB₂R-associated modulation of K⁺ channel function^[11]. In prefrontal cortical neurons, intracellular CB₂R are coupled to the G_{q11}-PLC-IP₃ pathway, which opens the Ca²⁺-dependent Cl⁻ channels, hyperpolarizes the cell membrane and results in neuronal inhibition^[40]. In hippocampal CA3/CA2 pyramidal neurons, activation of CB₂R triggers activation of the Na⁺/Bicarbonate co-transporter and causes a long-term neuronal hyperpolarization. This CB₂R activation occurs in a purely self-regulatory manner, robustly alters the input/output function of CA3 pyramidal cells, and modulates gamma oscillations *in vivo*^[17]. The relatively high expression of CB₂R in midbrain DA neurons suggests that they modulate a variety of important DA-associated behaviors^[41]. It has been reported that CB₂R modulate food intake, body weight^[42-45], depression^[46], anxiety^[22, 47], and schizophrenia-like behavior^[23, 48]. Recent reports emerging from several labs, including ours, have shown that brain CB₂R play a pivotal role in reducing cocaine, alcohol, and nicotine addiction^[49-51]. Collectively, these lines of evidence strongly suggest an important impact of CB₂R in the mesocorticolimbic system, as well as critical roles of CB₂R in various brain functions, including psychiatric, cognitive, and neurobiological activity.

Inducible feature of brain CB₂R and their modulation in neurological and psychiatric disorders

The most attractive property of the CB₂R is its inducible feature. Brain CB₂R are expressed at low levels under physiological conditions; however, in pathological conditions, such as neuropathic pain^[52], stroke^[53], traumatic brain injury^[54], neurodegenerative diseases^[55-57] or drug addiction^[58, 59], CB₂R expression up-regulates quickly and profoundly. This inducible feature lets CB₂R serve as a disease-associated target, and pharmacotherapeutic manipulation of CB₂R can treat diseases without side effects. For example, the mesocorticolimbic DA system is a key brain circuit implicated in a number of drug addictions. Alterations of the mesocorticolimbic DA circuit are the major cellular mechanisms to promote or prevent drug reward, dependence, and addiction. Emerging evidence has demonstrated that CB₂R modulate animal drug-seeking behaviors, including cocaine, alcohol, and nicotine^[49-51], suggesting a significant impact of brain CB₂R in animal drug reward, dependence, and addiction. Given the

lack of psychoactivity demonstrated by selective CB₂R agonists, CB₂R ligands have been developed as new candidates for the treatment of a variety of neurological and psychiatric disorders^[13, 60, 61], including pain^[62–65], neuroinflammation^[66], stroke^[67, 68], Alzheimer's disease^[69], Parkinson's disease, and Huntington's disease^[36, 70–75]. Three medicines that activate cannabinoid CB₁/CB₂ receptors are now used in clinics: Cesamet (nabilone), Marinol (dronabinol; Δ^9 -tetrahydrocannabinol [Δ^9 -THC]), and Sativex (Δ^9 -THC with cannabidiol). Additionally, a selective CB₂R agonist "Resunab™" has been designated by the FDA for a fast-track development program in a Phase II human clinical trial for scleroderma. However, significant attention is currently being directed at the possibility of developing medicines from compounds that can activate CB₂R at doses that induce little or no CB₁R activation. Accumulating lines of evidence have demonstrated that many of the adverse effects induced by mixed CB₁/CB₂ receptor agonists result from CB₁R rather than from CB₂R activation and that CB₂R-selective agonists have a number of important potential therapeutic applications^[60]. Therefore, we anticipate the emergence of new drugs that modulate CB₂R once a better understanding of the cannabinoid receptors is gained.

Limitation of brain CB₂R as a therapeutic target

The major challenge of how to selectively target brain CB₂R without affecting peripheral CB₂R remains, as CB₂R levels are much higher in peripheral tissues (*eg*, T-cells in the spleen) than in the brain. Thus, systemic exposure of CB₂R ligands to activate brain CB₂R will always activate peripheral CB₂R. We have two thoughts regarding this challenge: 1) Brain CB₂R are dramatically inducible, meaning they are up-regulated during disease conditions such as addiction, degeneration and inflammation. This pathology-associated increase significantly enhances the benefit to side-effect ratio^[76]. 2) Activation of brain CB₂R protects neurons from pathological conditions (*eg*, addiction, anxiety, stroke, epilepsy, pain) while also activating peripheral CB₂R (*eg*, T-cells), which may cause side effects in addition to central therapeutic effects. However, peripheral CB₂R activation will reduce the immune response and prevent an over-inflammatory reaction, which are beneficial for central protective effects. Therefore, the activation of peripheral CB₂R may not always induce side effects when brain CB₂R are activated, but rather, both central and peripheral CB₂R may work together to protect brain neurons from pathological alterations through both neuronal and immune mechanisms. Figure 1 shows a diagram of the impact of brain CB₂R distribution, function and disease.

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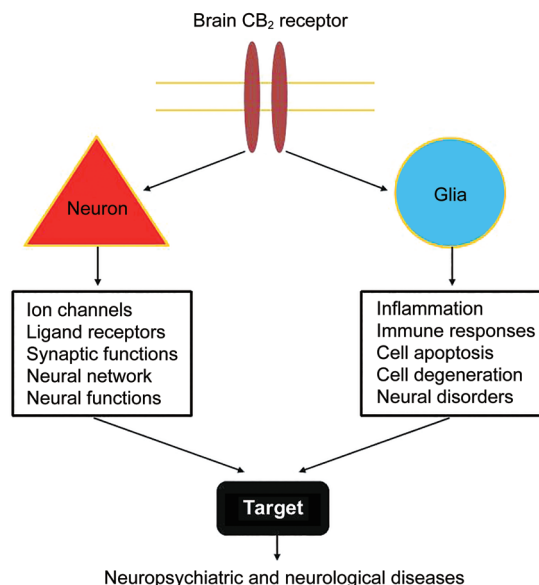


Figure 1. A diagram summarizing brain CB₂R expression and function and the association with neuropsychiatric and neurological diseases. CB₂R are expressed in brain neurons, where they participate in the modulation of a variety of neural functions and disorders. CB₂R are also expressed in brain glia cells, where they modulate immune function and neuroinflammatory responses. Therefore, brain CB₂R are an important target for the modulation of brain function and disease.

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