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Role of CB₂ receptors in neuroprotective effects of cannabinoids

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Abstract

CB₂ receptors, the so-called peripheral cannabinoid receptor type, were first described in the immune system, but they have been recently identified in the brain in healthy conditions and, in particular, after several types of cytotoxic stimuli. Specifically, CB₂ receptors were identified in microglial cells, astrocytes and, to a lesser extent, in certain subpopulations of neurons. Given the lack of psychoactivity demonstrated by selective CB₂ receptor agonists, this receptor becomes an interesting target for the treatment of neurological diseases, in particular, the case of certain neurodegenerative disorders in which induction/up-regulation of CB₂ receptors has been already demonstrated. These disorders include Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis and others. Interestingly, in experimental models of these disorders, the activation of CB₂ receptors has been related to a delayed progression of neurodegenerative events, in particular, those related to the toxic influence of microglial cells on neuronal homeostasis. The present article will review the evidence supporting that CB₂ receptors might represent a key element in the endogenous response against different types of cytotoxic events, and that this receptor type may be a clinically-promising target for the control of brain damage in neurodegenerative disorders.

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Key words: CB₂ receptors, CB₂ receptors agonists, cannabinoids, neurodegenerative disorders, local inflammation, reactive microglia

The CB₂ receptor within the cannabinoid signaling system

Studying the mechanism(s) of action of cannabinoids, the singular bi- and tricyclic compounds found in *Cannabis sativa*, several researchers identified in the 80s and 90s a novel intercellular signaling system, the so-called endocannabinoid system, that plays important modulatory functions in the brain and also in the periphery (for review, see Mackie, 2006). This identification started with the discovery of a membrane receptor, abundantly located in the brain although also present in the periphery, first called cannabinoid receptor and lately CB₁ receptor, whose activation is directly related to the psychoactivity typical of certain plant-derived cannabinoids (Pertwee, 2005). However, this is not the only receptor that may be activated by cannabinoids. In 1993, Munro and coworkers (Munro et al., 1993) cloned a second cannabinoid receptor type, the so-called CB₂ receptor, that is not involved in psychoactive effects of cannabinoids and, accordingly, it was initially found in the periphery, particularly in immune cells (for a recent review, see Raitio et al., 2005). This “peripheral cannabinoid receptor” is located in chromosome 1p36 in the human and encodes a protein of 360 amino acids with a 44% homology with the CB₁ receptor, although the homology is greater in the transmembrane domain (approximately 68%).

CB₂ receptor: pharmacological characteristics

The pharmacology of CB₂ receptors is in part similar to CB₁ receptors (e.g. most plant-derived and synthetic cannabinoid agonists activate CB₂ receptors), although the affinity and/or potency at which these agonists bind and/or activate CB₂ receptors present some interesting differences compared to CB₁ receptors (Pertwee, 2005; Fernández-Ruiz et al., 2007). This also happens in the case of endocannabinoid ligands, since anandamide (arachidonylethanolamide, AEA) was reported to be a weaker agonist for CB₁ receptors and not to significantly bind to CB₂ receptors (Mechoulam and Hanus, 2000; Sugiura et al., 2006), except in pathological conditions (Eljaschewitsch et al., 2006), whereas several studies suggested that 2-arachidonoylglycerol (2-AG) is an endogenous agonist for CB₂ receptors (Sugiura et al., 2006). It appears obvious that these pharmacological differences are the consequence, among others, of certain differences in chemical structures of cannabinoid agonists, differences that have been used to design selective synthetic CB₂ receptor agonists. This is the case of compounds such as JWH-133 and their analogs (Huffman, 2005), HU-308 (Hanus et al., 1999) and AM1241 (Malan et al., 2001), which represent novel tools to activate

selectively CB₂ receptors without the concomitant activation of the CB₁ receptor type. This represents an important goal since, although cannabinoids have a favorable drug safety profile, their use in the clinic is severely limited by the psychoactive effects elicited by most of cannabinoid agonists and mediated by the activation of CB₁ receptors. Therefore, an attractive alternative is to target CB₂ receptors selectively with the above-described agonists which are completely devoid of psychoactive effects, although they might exhibit other side effects such as immune suppression (Pertwee, 2005). Their potential for the treatment of certain neurological diseases is presently being examined in multiple preclinical studies, including several neuroinflammatory/neurodegenerative disorders in which CB₂ receptor agonists might serve to delay the progression of neuronal damage (Fernández-Ruiz et al., 2007, for a recent review). On the other hand, selective antagonists for the CB₂ receptor are also currently available, and constitute important tools to elucidate the involvement of this receptor type in specific cellular functions (for review, see Pertwee, 2005; Fernández-Ruiz et al., 2007).

CB₂ receptor: tissue and cell distribution

As mentioned above, the first studies that explored the tissue and cell distribution of CB₂ receptors indicated that this cannabinoid receptor type was exclusively present in tissues and cells of the immune system (e.g. spleen macrophages, tonsils, B cells and natural killer cells, monocytes, neutrophils and T cells; Howlett et al., 2002; Pertwee, 2005; Fernández-Ruiz et al., 2007, for review), being absent from the CNS (Lynn and Herkenham, 1994), in notable contrast with the well-known distribution of CB₁ receptors. Further studies suggested that, although absent from the CNS in normal conditions, this receptor might be induced in glial cells, in particular reactive microglia, in response to different damaging conditions associated with local inflammatory events (see Fernández-Ruiz et al., 2007, for review). Lastly, recent studies have proposed that CB₂ receptors may be present in the brain even in healthy conditions (Onaivi et al., 2006), despite this issue has remained controversial due to uncertainty of experimental approaches used or of some methodological tools available (e.g. anti-CB₂ antibody). These studies identified CB₂ receptors in glial cells, including microglia and astrocytes (Stella, 2004; Nuñez et al., 2004; Maresz et al., 2005), neural (Palazuelos et al., 2006) and oligodendroglial (Molina-Holgado et al., 2002) progenitors, and certain neuronal subpopulations (Skaper et al., 1996; Ross et al., 2001; Stander et al., 2005; Wotherspoon et al., 2005; Van Sickle et al., 2005; Beltramo et al., 2006) in different brain structures of

various species, including human samples (Nuñez et al., 2004) and using either *in vivo* or *in vitro* approaches.

CB₂ receptor: signaling mechanisms

CB₂ receptors belong to the seven-transmembrane domain, G-protein-coupled receptor class (Howlett et al., 2002). They are coupled to G_{i/o} proteins, so that their activation, in various cell types expressing the receptor either naturally or heterologously, is associated with classic intracellular responses: (i) the inhibition of adenylyl cyclase and the cAMP/protein kinase A (PKA)-dependent pathway (Howlett et al., 2002); and (ii) the stimulation of mitogen-activated protein kinase (MAPK) cascades, specifically the extracellular signal-regulated kinase (ERK) (Bouaboula et al., 1996; Carrier et al., 2004; Palazuelos et al., 2006) and the p38 MAPK cascades (Gertsch et al., 2004; Herrera et al., 2005). In addition, the activation of CB₂ receptors has been also linked to the stimulation of additional intracellular pathways including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Molina-Holgado et al., 2002; Samson et al., 2003; Palazuelos et al., 2006), which has been associated with pro-survival effects, and the *de novo* synthesis of the sphingolipid messenger ceramide (Sánchez et al., 2001; Carracedo et al., 2006a and 2006b), which has been linked with the pro-apoptotic effects of cannabinoids.

CB₂ receptor and neuroprotection

Most of physiological functions associated with the CB₂ receptor deal with different types of immunological effects given the predominance of this receptor type over the CB₁ receptor in immune tissues. However, its recent description in certain brain regions allowed relate this receptor type to those neurobiological processes located in those region, for example, the control of pain, brain reward, emotion and others (Onaivi et al., 2006, for review). An important point is the implication of the CB₂ receptor in processes related to the control of proliferation (Carrier et al., 2004; Palazuelos et al., 2006), differentiation (Sánchez et al., 2001; Alberich Jorda et al., 2004; Palazuelos et al., 2006) and survival (Sánchez et al., 2001) of neural cells. That the CB₂ receptor plays a role in these key cell processes is the basis for the proposal that selective agonists of this receptor type may act on “two sides of a coin” by providing cytoprotection of healthy neural cells or by eliciting apoptosis of tumoral cells (see Fernández-Ruiz et al., 2007, for a recent review). This review will focus only in the first of

these two properties, namely, their capability to arrest/delay brain damage in different neurodegenerative disorders, particularly in those that exhibit an important local inflammatory component associated with brain injury. This capability adds to other neuroprotective mechanisms elicited by different elements of the cannabinoid signaling system, in particular the CB₁ receptor that has been associated with inhibition of glutamate release, decrease of cytosolic free Ca²⁺ concentration and vasodilation, effects that are overall capable to increase neuronal survival (see van der Stelt and Di Marzo, 2005; Fernández-Ruiz et al., 2005, for review). This potential, together with other cannabinoid receptor-independent properties (e.g., blockade of NMDA receptors, antioxidant activity; see Fernández-Ruiz et al., 2005, for review), allows cannabinoids to protect neurons from death and, accordingly, to be beneficial as novel therapies for acute brain injury (cerebral ischemia and trauma) and for chronic neurodegenerative disorders [e.g. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS)].

Up-regulation/induction of CB₂ receptors in pathological brain

Despite the present evidence indicating that CB₂ receptors are present in the normal CNS of rodents and humans (Onaivi et al., 2006), it is likely that these receptors experience a marked elevation in discrete brain regions after pathological neuroinflammatory insults (Fernández-Ruiz et al., 2007). This might include either their up-regulation in cells that naturally express CB₂ receptors (e.g. astrocytes; Stella, 2004) or their induction in cells that are recruited and activated in response to damaging stimuli (e.g. reactive microglial cells; Fernández-Ruiz et al., 2005 and 2007). This type of response has been observed in those structures undergoing neuronal damage: (i) in the rat brain following stroke (Ashton et al., 2007); (ii) in a rat model of HD (Fernández-Ruiz et al., 2005 and 2007); (iii) in SIV encephalitis (Benito et al., 2005) and HIV dementia (Romero et al., unpublished observations); (iv) in patients with Down's syndrome (Nuñez et al., 2007); (v) in the periphery of senile plaques in AD patients (Benito et al., 2003) and in an experimental model of AD in rats (Esposito et al., 2007); (vi) in a rat model of neuropathic pain (Zhang et al., 2003); (vii) in patients with MS (Yiangou et al., 2006; Benito et al., 2007) and in an experimental model of MS in mice (Maresz et al., 2005); and (viii) also in patients with ALS (Yiangou et al., 2006). This up-regulatory response of CB₂ receptors would be paralleled in some cases by equivalent responses of CB₁ receptors and endocannabinoid ligands (Fernández-Ruiz et al., 2005 and 2007). Therefore, one may

assume that the activation of the cannabinoid signaling system represents an endogenous response of the brain to maintain nerve cell homeostasis and to reduce the injury associated with conditions of excitotoxicity, inflammation, trauma, infection and other types of neurotoxic stimuli.

Neuroprotective action of CB₂ receptors

In vivo or *in vitro* studies, using selective CB₂ receptor agonists (e.g. HU-308, AM1241) or reversion with selective CB₂ receptor antagonists (e.g. SR144528, AM630) of effects of non-selective agonists (e.g. WIN55,212-2), have demonstrated that this receptor may represent a pharmacologically valuable tool to protect neurons from death in a variety of acute and chronic neurodegenerative disorders (see Fernández-Ruiz et al., 2007, for review). This is the case of studies conducted in experimental models of perinatal hypoxia-ischemia (Fernández-López et al., 2006), focal ischemis/reperfusion (Zhang et al., 2007), HD (Fernández-Ruiz et al., 2007), AD (Ramírez et al., 2005), ALS (Kim et al., 2006) and MS (Arévalo-Martín et al., 2003). However, this is not the case for PD in which only antioxidant cannabinoids, but not CB₂ receptor agonists, provided neuroprotection (Lastres-Becker et al., 2005; García-Arencibia et al., 2007). In those neurodegenerative disorders where the activation of CB₂ receptors is neuroprotective, this effect is, as expected, importantly linked to the recruitment, activation and migration of microglial cells to the sites of lesion and with the function of these cells on neuronal homeostasis (Eljaschewitsch et al., 2006; Fernández-Ruiz et al., 2007; see also the following section). In fact, CB₂ receptor immunoreactivity has been identified in discrete subpopulations of microglia at the lesioned brain structures in most of these disorders, as has been detailed in the above section (Fernández-Ruiz et al., 2007, for review).

Control of glial-mediated effects by CB₂ receptors

Given that CB₂ receptors that provide neuroprotection in neurodegenerative disorders (see above section) seem to be those that are induced/up-regulated in glial cells in response to damaging stimuli, it appears likely to assume that their function within the endogenous protection against these stimuli would be related to limiting the toxic influence of microglia on neuronal homeostasis, to enhancing the protection exerted by astrocytes, or both. For instance, astrocytes have been reported to function as “neuroprotective cells” by generating pro-survival factors (e.g. neurotrophins) or metabolic substrates (e.g. ketone bodies, lactate)

for neurons (see Chen and Swanson, 2003 for review). CB₂ receptors have been identified in these glial cells at lesioned sites (Fernández-Ruiz et al., 2005). Therefore, it is possible that these receptors might enhance the generation of pro-survival factors or the support of metabolic substrates (see Figure 1 for an overview), but this possibility has not been determined yet. By contrast, microglial cells are recruited in response to brain injury and migrate at lesioned sites where they might play a primary “protective” function but that secondarily becomes toxic for neurons. It has been proposed, and demonstrated in a series of elegant studies (Puffenbarger et al., 2000; Facchinetti et al., 2003; Stella, 2004), that, in microglia, the up-regulatory response of CB₂ receptors would be aimed at controlling the processes of proliferation, differentiation and migration of these cells (Walter et al., 2003; Carrier et al., 2004), as well as at limiting the magnitude of neurotoxic response exerted by microglial cells (Fernández-Ruiz et al., 2007). According to this, the activation of CB₂ receptors would reduce the generation of neurotoxic factors, such as nitric oxide, proinflammatory cytokines and reactive oxygen species, by these reactive glial cells (see Fernández-Ruiz et al., 2007, for a recent review and Figure 1 for an overview). This type of response has been observed in animal models of perinatal hypoxia-ischemia (Fernández-López et al., 2006) and HD (Fernández-Ruiz et al., 2005) where the activation of CB₂ receptors reduced the release of proinflammatory factors, including nitric oxide, TNF α , IL-1 and IL-6. By contrast, some of the antiinflammatory effects of cannabinoid receptor activation could be mediated by enhancing the action of anti-inflammatory molecules, such as IL-1ra (Molina-Holgado et al., 2003; Fernández-Ruiz et al., 2005 and 2007, for recent reviews).

Closing remarks

The studies reviewed here strongly support that brain CB₂ receptors are important players in the control of neuroinflammatory events associated with conditions of brain damage of different etiologies. In pathological conditions, CB₂ receptors are induced in cells that do not contain these receptors in healthy conditions (e.g. reactive microglia) or are up-regulated in cells that do contain a small number of these receptors. These responses are likely part of an endogenous mechanism of defense against a variety of brain-damaging insults, where they appear associated with equivalent responses in other elements of the cannabinoid signaling. Through the activation of these receptors, either in reactive microglial cells or in astrocytes, cannabinoid agonists might limit the toxic influence of microglia on neuronal homeostasis or potentiate the metabolic support exerted by astrocytes. Both effects, occurring independently

or concomitantly, may enhance the possibilities of neuronal survival, so that this receptor represents a clinically-promising target for delaying/arresting the progression of neurodegeneration in acute and, in particular, in chronic neurological disorders.

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Figure 1. Role of glial cells in CB₂ receptor-mediated neuroprotection. CB₂ receptors are located in reactive microglia and astrocytes and they play a role in the endogenous response against neurodegenerative/neuroinflammatory stimuli.

