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Cannabinoid CB_2 receptor: a new target for controlling neural cell survival?

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Two types of cannabinoid receptor have been cloned and characterized. Whereas CB_1 receptors are ubiquitously expressed in neurons of the CNS, CB_2 receptors have been thought to be absent from the CNS. Recent data now question this notion and support the expression of CB_2 receptors in microglial cells, astrocytes and even some neuron subpopulations. This discrete distribution makes CB_2 receptors interesting targets for treating neurological disorders because CB_2-selective agonists lack psychoactivity. Here, we review evidence supporting the idea that CB_2 receptors are implicated in the control of fundamental neural cell processes, such as proliferation and survival, and that their pharmacological manipulation might be useful for both delaying the progression of neurodegenerative disorders and inhibiting the growth of glial tumors.

Introduction: the cannabinoid signaling system

The great increase in cannabinoid research in recent years has been a direct consequence of the discovery of the 'endogenous cannabinoid system' – a new intercellular communication network [1]. The endogenous cannabinoid system comprises at least two types of G protein-coupled receptor, called the cannabinoid CB_1 and CB_2 receptors, that are activated by a family of endogenous arachidonic acid derivatives, the endocannabinoids, and by Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal active ingredient of the hemp plant *Cannabis sativa* L. The action of endocannabinoids at their receptors is terminated by an endogenous mechanism of deactivation that involves a membrane transport system and at least two degradation enzymes [1,2].

Endocannabinoids are active at various sites of the body, such as the cardiovascular and immune systems, but their best-described role is to provide neuromodulatory functions in the mammalian brain. Thus, by acting as retrograde messengers at various synapses, endocannabinoids participate in controlling processes such as motor activity, memory and learning, appetite, emesis, nociception and some motivational responses [1,2]. In addition, these ligands and their receptors control the survival or death decision of neural cells [3], such that the pharmacological manipulation of this system might provide either neuroprotective [4] or proapoptotic [5] effects. Although seemingly opposite, these two effects become complementary if one assumes that brain-protective pharmacological approaches should focus mainly on increasing the survival of 'healthy' nerve cells and/or on inducing the death of non-desired (e.g. transformed) neural cells.

Although cannabinoids have a favorable drug safety profile, their use in the clinic is severely limited by their psychoactivity. Because the unwanted psychotropic effects of marijuana-derived cannabinoids are mediated largely or completely by neuronal CB_1 receptors, the most obvious alternative possibility is to target CB_2 receptors alone. Selective agonists for this receptor type have been developed and are completely devoid of psychoactivity, although they might have other side-effects such as immune suppression [6]. Although the necessary clinical trials are pending, preclinical studies support the idea that these ligands are promising tools for treating diseases associated with alterations of neural cell survival, owing to their capacity to delay the progression of neurodegenerative disorders [7] and to inhibit the growth of glial tumors [5].

On the basis of this evidence, here we focus on the most recent data on the presence and function of CB_2 receptors in the CNS under normal conditions and on their potential role in neurodegeneration, neuroinflammation and neural cell apoptosis.

Biochemistry, pharmacology and distribution of the CB_2 receptor

The gene encoding the human cannabinoid CB_2 receptor was cloned in 1993 (recently reviewed in Ref. [8]). It is located in chromosome 1p36 and encodes a protein of 360 amino acids with an overall homology to CB_1 receptors of 44%, although this homology rises to 68% when only the transmembrane regions are compared. It belongs to the seven-transmembrane-domain, G-protein-coupled receptor class [9], and can modulate various signal transduction pathways involved in controlling cell proliferation, differentiation and survival (Figure 1). Thus, by coupling to $G_{i/o}$ proteins, the CB_2 receptor inhibits adenylyl cyclase and the cAMP pathway in various types of cell expressing

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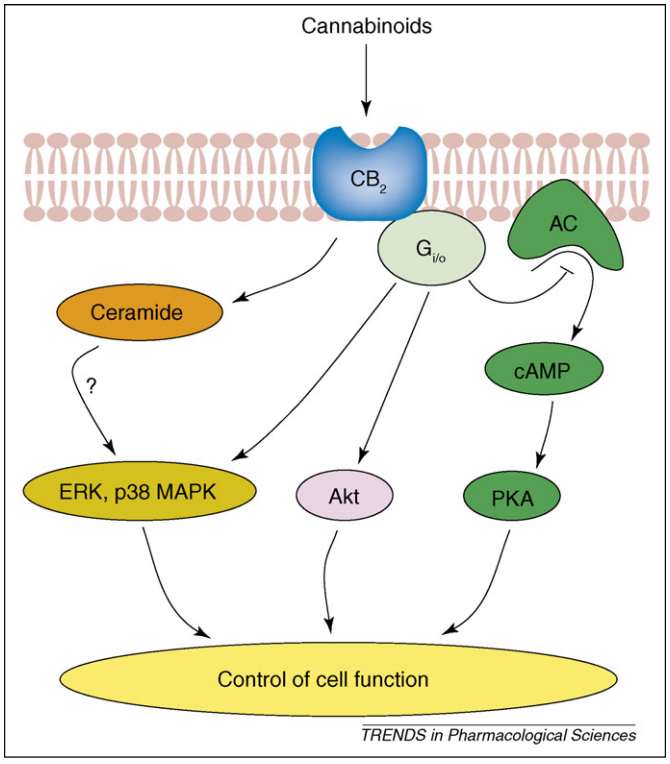

Figure 1. Signaling pathways coupled to the CB_2 receptor. Cannabinoids exert their effects by binding to specific receptors. The $G_{i/o}$-protein-coupled CB_2 receptor signals to several different cellular pathways, including the adenylyl cyclase (AC), cAMP and protein kinase A (PKA) pathway; MAPK cascades (ERK, p38 MAPK); the Akt pathway; and a pathway for *de novo* synthesis of ceramide. For clarity, cross-talk among the different pathways has been omitted.

the receptor either naturally or heterologously [9], and stimulates mitogen-activated protein kinase (MAPK) cascades: specifically, the extracellular-signal-regulated kinase (ERK) cascade in Chinese hamster ovary cells expressing recombinant CB_2 receptors [10], rat RTMGL1 microglial cells [11] and mouse neural progenitors [12]; and the p38 MAPK cascade in human monocytes [13] and leukemia cells [14]. Pharmacological stimulation of the CB_2 receptor also activates the pro-survival phosphatidylinositol 3-kinase and Akt (PI3K–Akt) pathway in rat oligodendroglial cells [15], rat RBL2H3M1 mast cells [16] and mouse neural progenitors [12]. In addition, CB_2 receptor engagement enhances *de novo* synthesis of the sphingolipid messenger ceramide in rat C6 glioma cells [17], human U87 astrocytoma cells [18], and non-neural cells such as human MiaPaCa2 pancreatic cancer cells [19].

Most plant-derived and synthetic cannabinoid agonists activate CB_2 receptors, although their affinity for this receptor type shows some interesting differences as compared with CB_1 receptors [6] (Table 1). With regard to endocannabinoid ligands, several studies support the notion that 2-arachidonoylglycerol is the true endogenous agonist for CB_2 receptors [20]. Anandamide (also known as arachidonoylethanolamide or AEA), the first endocannabinoid to be isolated and characterized, seems to be a weaker agonist for CB_1 receptors and seems not to bind significantly to CB_2 receptors [20,21]. Anandamide, however, has been recently linked to activation of the CB_2 receptor in pathological conditions [22]. Recent work has facilitated the synthesis of compounds such as JWH-133 and its analogs [23], HU-308 [24] and AM1241 [6,8,25] (Table 1), which represent novel tools with which to activate CB_2 receptors selectively without concomitant stimulation of the psychoactive CB_1 receptors. Selective antagonists for the CB_2 receptor are also currently available and will be important tools for elucidating the involvement of this receptor type in specific cellular functions (for review, see Refs [6,9]) (Table 1).

With regard to the tissue and cell distribution of CB_2 receptors, initial studies supported the idea that this cannabinoid receptor type is expressed exclusively in peripheral tissues, particular in the marginal zone of the spleen [2,6]. Shortly after, CB_2 receptors were reported to be expressed in many other tissues and cell types of the immune system [9]. These and other [26] seminal studies indicated that CB_2 receptors are absent from the CNS, in notable contrast to the well-known distribution of CB_1 receptors. More recent evidence has shown, however, that CB_2 receptors are present in both cultured neural cells and the nervous system of several mammals such as rodents, monkeys and humans under normal conditions. For example, CB_2 receptors are expressed in glial cells (microglia and astrocytes) [27], and neural [12] and oligodendroglial [15] progenitors in culture, and in microglia [28] and neural progenitors [12] in normal mouse brain *in vivo*.

The existence of CB_2 or CB_2-like receptors in neuron subpopulations is becoming a hot topic in this field. First, expression of CB_2 receptor mRNA was identified in neuronal elements of the mouse cerebellum as a consequence of an excitotoxic insult [29]. The presence of these receptors in several neuronal cell lines [30] and sensory neurons [31–33] was subsequently reported, and a recent study has shown that CB_2 receptors are expressed in mouse, rat and ferret brainstem neurons [34]. Albeit with obvious

Table 1. Molecular and pharmacological characteristics of CB_2 receptors

Characteristics	CB_2 receptors	Comparison with CB_1 receptors
Tissue and cell localization	CNS (microglia and astrocytes) Immune tissues and cells Other tissues (e.g. retina)	CB_2 receptors are significantly less concentrated in neurons, where CB_1 receptors are ubiquitously distributed
Endogenous agonists	2-Arachidonoylglycerol	Anandamide is more active at CB_1 receptors
Other agonists	Selective: HU-308, JWH-133, AM1241 Non-selective: Δ^9-THC and cannabinol have equivalent affinity; WIN55 212–2, HU-210	Δ^9-THC binds to CB_1 receptors with a tenfold higher affinity than cannabinol
Antagonists	SR144528, AM630	Most of antagonists for each receptor type are selective
Signaling mechanisms	Inhibition of adenylyl cyclase Activation of MAPK cascades Activation of the PI3K–Akt pathway	CB_1 receptors are also coupled to different ion channels

inherent limitations, the use of macaque and human samples has shown that CB_2 receptors are expressed *in vitro* by astrocytes and microglial cells and *in vivo* by perivascular microglia [35] – that is, the subpopulation of microglia that surrounds cerebral blood vessels. These microglial cells have the same immune origin as resident microglia, but are subject to a faster turnover; thus, they might have crucial roles in specific processes such as viral entry to the CNS.

The CB_2 receptor and the cell death or survival decision

The CB_2 receptor has been implicated in controlling the proliferation, differentiation and survival of both neural and non-neural cells. For example, activation of CB_2 receptors in rat RTMGL1 microglial cells [11] and mouse neural progenitors [12] promotes cell proliferation. Moreover, an inverse relation between expression of the CB_2 receptor and the stage of cell differentiation is evident in neural cells (from neural progenitors to mature neurons and neuroglial cells [12]) and in B cells (from virgin B cells to centroblasts [36]), which suggests that this receptor might function as a 'cell de-differentiation signal' by favoring a non-differentiated, proliferative state. In line with this notion, expression of the CB_2 receptor increases with the de-differentiation (i.e. with increased malignancy) of glial [17] and breast [37] tumors, and activation and overexpression of the CB_2 receptor block neutrophil cell differentiation [38]. By contrast, studies conducted in glioma or astrocytoma cells [17] and in various non-neural cancer cells [5,19,37] show that activation of the CB_2 receptor induces apoptosis and inhibits tumor growth in host mice. These varying actions of the CB_2 receptor would enable selective agonists of this receptor type to act on 'two sides of a coin' by providing cytoprotection [4] or by eliciting apoptosis [5].

One side of the coin: CB_2 receptors and neuroprotection

Substantial *in vivo* and *in vitro* results show that cannabinoids protect neurons from death [4,7,39]. This neuroprotection might be relevant for the treatment of both acute brain injury (e.g. cerebral ischemia and trauma) and chronic neurodegenerative disorders (e.g. Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis). Cannabinoid neuroprotection involves a myriad of molecular and cellular mechanisms. Some of these mechanisms are mediated by the CB_1 receptor (e.g. inhibition of glutamate release, decrease in cytosolic free Ca^{2+} concentration, and vasodilatation), whereas others are independent of CB receptors (e.g. blockade of NMDA receptors and antioxidant activity) [4,7,39].

Recent studies have now implicated CB_2 receptors in the neuroprotective activity of cannabinoids, mainly through a series of glia-dependent anti-inflammatory actions [7]. It is important to note that, in addition to excitotoxicity, mitochondrial failure and oxidative stress, the activation of glial cells – in particular that of microglial cells – is a primary process for local inflammatory events and therefore for the pathogenesis of neurodegenerative disorders. Thus, as discussed below in more detail, CB_2 receptors are upregulated in reactive microglia and astrocytes in response to stimuli that provoke local inflammatory processes, such as those occurring in several chronic neurodegenerative disorders.

In microglia, this upregulatory response is thought to be aimed at controlling the production of neurotoxic factors, such as nitric oxide, proinflammatory cytokines and reactive oxygen species, by these reactive glial cells (for review, see Ref. [7]) (Figure 2). Astrocytes might also contribute to this neuroprotective effect by generating prosurvival factors or metabolic substrates for neurons, but the function of CB_2 receptors in these responses has not been determined. In response to equivalent degenerative stimuli, the action

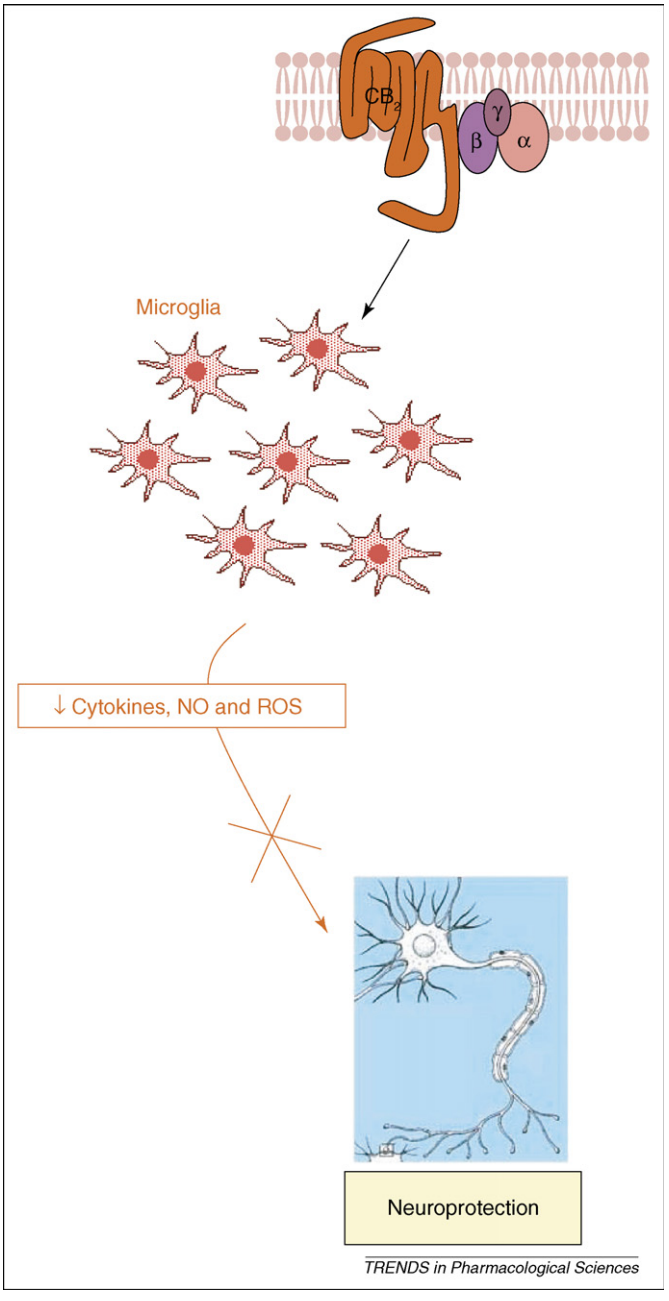

Figure 2. Role of microglial cells in neuroprotection mediated by the CB_2 receptor. CB_2 receptors are induced or upregulated in reactive microglial cells in response to degenerative or inflammatory stimuli. The activation of CB_2 receptors might control the production of different cytotoxic factors such as cytokines, nitric oxide (NO) or reactive oxygen species (ROS) by these cells, thereby increasing neuronal survival.

mediated by CB_2 receptors would be concomitant with that reliant on CB_1 receptors and endocannabinoid ligands [7,39]. We might, therefore, speculate that activation of different elements of the endocannabinoid signaling system represents an endogenous response of the brain to maintain nerve cell homeostasis and to reduce the injury associated with excitotoxicity, inflammation, trauma, infection and other types of cytotoxic stimulus.

Upregulation of CB_2 receptors in pathological brain

As mentioned above, CB_2 receptors are present in the CNS of rodents and humans under normal conditions. Of interest, expression of CB_2 increases markedly, mostly in microglial elements of discrete brain regions, after pathological neuroinflammatory insults [7]. For example, CB_2 receptors are upregulated both in reactive microglial cells located in the periphery of senile plaques in individuals with Alzheimer's disease [40] and in the lesioned striatum in a rat model replicating human Huntington's disease pathology [7]. They are also upregulated in SIV encephalitis [41] and in individuals affected with HIV encephalitis, Down's syndrome and multiple sclerosis (J. Romero *et al.*, unpublished). In addition, data from SIVE encephalitis [41] and HIV encephalitis (J. Romero *et al.*, unpublished) specimens show that T lymphocytes expressing the CB_2 receptor are present in infiltrative areas, mostly at perivascular locations, suggesting that CB_2 receptors might be involved in lymphocyte infiltration into the CNS.

Neuroprotective action of CB_2 receptors

A beneficial effect of CB_2 receptor activation has been shown in different *in vivo* and *in vitro* models of acute and chronic neurodegenerative disorders. For example, the neuroprotective effect of the non-selective agonist WIN-55 212–2 in a rat perinatal model of hypoxia–ischemia is mediated, at least in part, by CB_2 receptors [42], which are thought to be stimulated in response to the ischemic insult. Equivalent data have been obtained in animal models of other neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis and multiple sclerosis (see below), but not in Parkinson's disease, where only antioxidant cannabinoids, and not CB_2 receptor agonists, have been found to provide neuroprotection [7,43].

In Huntington's disease, the plant-derived cannabinoid Δ^9-THC reduces the death of striatal projection neurons [44]. We have recently found that this effect might be mediated by CB_2 receptors [7]: immunofluorescence analyses have revealed an increase in the expression of CB_2 receptors in discrete subpopulations of microglia and astroglia at the lesioned striatum. It is possible that an equivalent situation occurs in Alzheimer's disease, because WIN-55 212–2 exerts neuroprotection in a rat model of the disease, an effect that can be reproduced in co-cultures of microglia and neurons by selectively targeting CB_2 receptors [45]. A similar neuroprotective role of cannabinoid-induced microglial deactivation has been recently put forward by another group [22].

In a mouse model of amyotrophic lateral sclerosis, administration of the selective CB_2 receptor agonist AM1241 slows the signs of disease progression [46]. With regard to multiple sclerosis, work by two groups has characterized the effects of cannabinoids mediated by CB_1 and CB_2 receptors on spinal inflammation, demyelination and axonal injury in mouse models of the disease [47,48].

Control of glial activation by CB_2 receptors

It is widely accepted that CB_2 receptor activation contributes to a decrease in the *in vitro* production of proinflammatory molecules in several neural cell types such as rat microglial cells [49,50], primary mouse astrocytes [51,52], human microglial and THP-1 cells [27], and human astrocytes [53]. Activation of CB_2 receptors also reduces the release of proinflammatory factors in animal models of perinatal hypoxia–ischemia [42] and Huntington's disease [7]. Of the proinflammatory molecules that seem to be under control of the CB_2 receptor, nitric oxide, tumor-necrosis factor-α, interleukin 1 (IL-1) and IL-6 are the most relevant. In addition, some of the anti-inflammatory effects of cannabinoid receptor activation could be mediated by enhancing the action of anti-inflammatory molecules such as IL-1ra [7,54]. Taken together, these data are consistent with the notion that CB_2 receptor stimulation has significant anti-inflammatory activity in glial cells, thereby constituting one of the most exciting areas of interest in the neuropharmacology of cannabinoid compounds.

In addition, the rat microglial cell line RTMGL1 produces 2-arachidonoylglycerol, which induces cell proliferation through activation of the CB_2 receptor [11], and CB_2 receptor activation leads to an enhancement of microglial recruitment and migration after pathological stimulation with ATP [55]. Anandamide has recently been reported to be produced by microglial cells [22]. Thus, the endocannabinoid system would modulate microglial responses mostly under stress conditions such as those present in neurodegenerative processes.

The other side of the coin: CB_2 receptors and glioma cell death

Investigations carried out during the past few years have shown that administration of cannabinoids inhibits the survival of various tumor cells in culture and, more importantly, curbs the growth of different models of tumor xenografts in rats and mice [5]. This antitumor effect has been mainly studied in tumors of the CNS, specifically gliomas [56].

Experiments conducted with ligands selective for either CB_1 or CB_2 receptors in rat C6 glioma cells [17,57], several human astrocytoma cell lines [18] (G. Velasco *et al.*, unpublished) and cells obtained from human astrocytoma biopsies [17] support the concept that CB_2 receptor stimulation is involved in cannabinoid antitumor activity *in vitro* and in mice inoculated with tumor xenografts *in vivo*, consistent with observations in experimental models of skin [58] and pancreatic [19] carcinomas. These and other studies have also provided substantial evidence implicating at least two mechanisms in cannabinoid antitumor activity: induction of tumor cell apoptosis, and inhibition of tumor angiogenesis (Figure 3a).

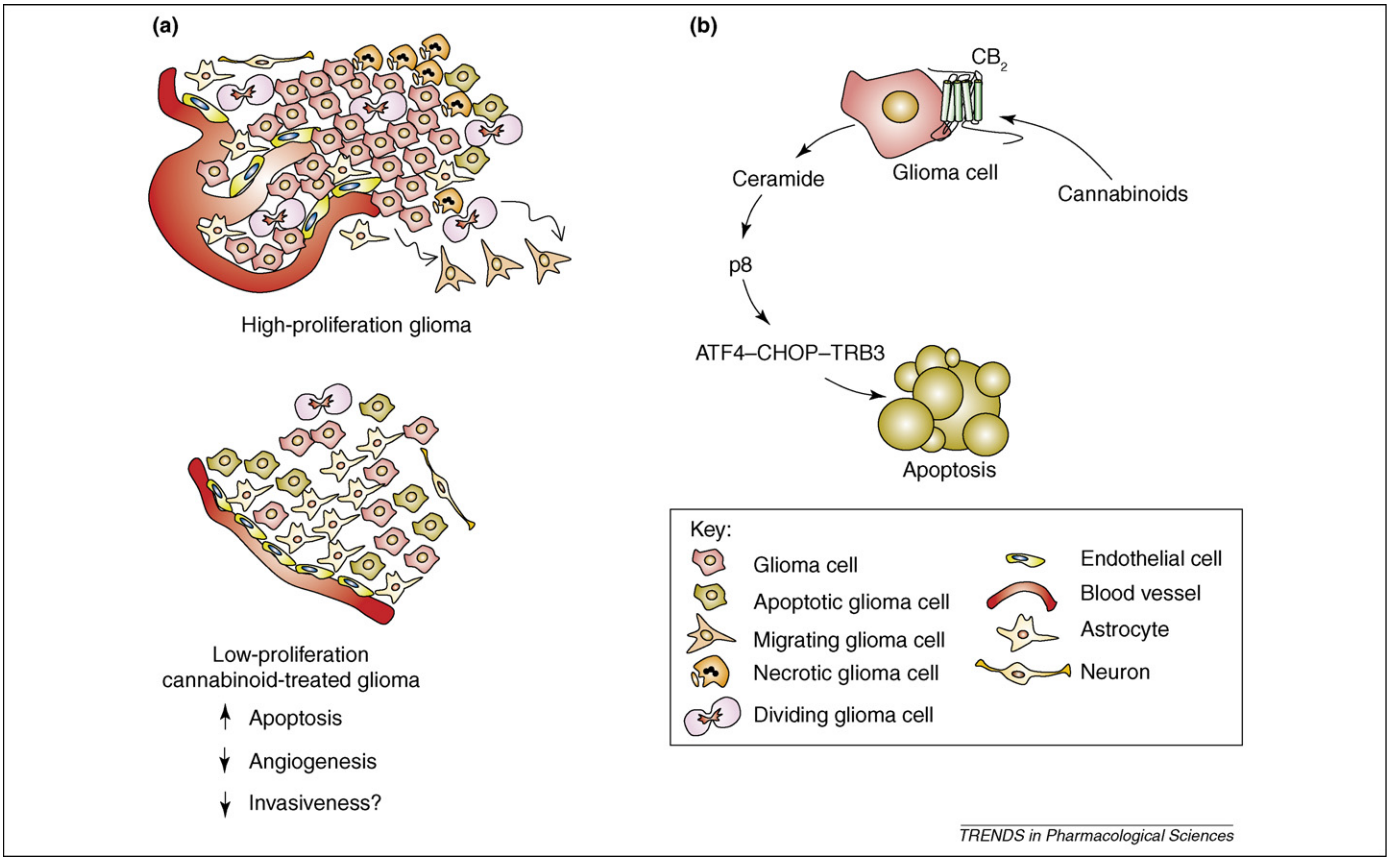

Figure 3. Antitumor action mediated by the CB_2 receptor in gliomas. **(a)** Actively growing gliomas are characterized by high cell proliferation, angiogenesis and invasiveness. Cannabinoids might mediate their antitumor action in gliomas by inducing tumor cell apoptosis, and by inhibiting tumor angiogenesis and perhaps tumor invasiveness. **(b)** Mechanism of cannabinoid-induced apoptosis of glioma cells. Engagement of cannabinoid receptors enhances ceramide synthesis *de novo*, which leads to induction of p8, followed by upregulation of the stress-related proteins ATF4, CHOP and TRB3.

Induction of tumor cell apoptosis

Pharmacological activation of CB_2 receptors on glioma and/or astrocytoma cells induces apoptosis *in vitro* and *in vivo* [5,18,57]. Different experimental strategies have shown that this process relies, at least in part, on induction through the CB_2 receptor of *de novo* synthesis of ceramide – a sphingolipid second messenger that can induce apoptosis, cell-cycle arrest and stress responses in various cell types [5]. It is worth noting that cellular ceramide has been inversely related to malignant progression and to a poor prognosis of human astrocytomas. Thus, low-grade astrocytomas have a higher ceramide content than do high-grade astrocytomas [56].

Some of the downstream effectors of ceramide-mediated apoptosis have been recently characterized in cannabinoid-treated glioma cells *in vitro* and *in vivo* [18] (Figure 3b). For example, ceramide that has been synthesized *de novo* induces the rapid expression of p8 (also known as candidate of metastasis 1), a member of the HMG-I/Y family of transcription factors, which in turn upregulates two endoplasmic reticulum stress-response-related transcription factors – namely, activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP; also known as DDIT3). The last two proteins operate in concert to enhance expression of the stress-regulated pseudokinase Tribbles homolog 3 (TRB3; also known as NIPK and SKIP3). The action of this and other pro-apoptotic proteins might converge – by a hitherto unclear mechanism – in the mitochondria to trigger the intrinsic apoptotic pathway and the activation of executioner caspases.

Of note, under comparable experimental conditions cannabinoids do not affect the activity of the p8–ATF4–CHOP–TRB3 pathway or the survival of normal, non-transformed cells of glial [18] and pancreatic [19] origin, supporting the current notion that cannabinoids regulate cell survival pathways differently in cancer and non-cancer cells [5].

Inhibition of tumor angiogenesis

To grow beyond minimal size, tumors must generate a new vascular supply (angiogenesis) for the purposes of cell nutrition, gas exchange and waste disposal; as a result, blocking the angiogenic process constitutes one of the most promising antitumor approaches that is currently available. Immunohistochemical and functional analyses in mouse models of glioma have shown that administration of cannabinoids turns the vascular hyperplasia characteristic of actively growing tumors to a pattern of blood vessels characterized by small, differentiated and impermeable capillaries [59]. This change is due, at least in part, to inhibition of the vascular endothelial growth factor (VEGF) pathway through the CB_2 receptor [60], an observation that is also supported by data from skin carcinomas [58].

Interestingly, pharmacological inhibition of ceramide synthesis *de novo* abrogates the antitumor and antiangiogenic effects of cannabinoids *in vivo*, and decreases the

production of VEGF by glioma cells *in vitro* and by gliomas *in vivo* [60], supporting the idea that ceramide has a general role in cannabinoid antitumor action. Other factors, such as the inhibition of vascular endothelial cell migration and survival, and the downregulation of matrix metalloproteinase-2, could also contribute to the inhibition of glioma angiogenesis and invasiveness mediated by the CB_2 receptor [59].

Concluding remarks and future perspectives

Recent data support the concept that brain CB_2 receptors have important functions in attenuating neuroinflammatory processes and in inhibiting the survival of transformed neural cells. These receptors are upregulated in pathological conditions, presumably as part of an endogenous mechanism of defense against various brain-damaging insults; however, further work is required to obtain full characterization of the possible roles that these receptors have in those processes.

Essential issues to be addressed include (i) the existence and functionality of neuronal subpopulations of CB_2 or CB_2-like receptors; (ii) a comprehensive description of the mechanisms of signal transduction that are coupled to CB_2 receptor activation; (iii) the possible pathophysiological functions of this receptor in functionality of the blood–brain barrier; (iv) the molecular cascades triggered by CB_2 receptor activation for controlling cytokine production; and (v) the therapeutic potential derived from pharmacological manipulation of CB_2 receptors in acute and chronic neurodegeneration and in brain tumors. In this respect, the preclinical studies reviewed here indicate that CB_2 receptor activation provides neuroprotection and elicits apoptosis of brain tumor cells; this supports the need for a future clinical testing of compounds that selectively target this cannabinoid receptor type in patients affected by neurodegeneration or brain tumors.

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