Are microglial CB_2 cannabinoid receptors involved in the etiology of schizophrenia?

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Abstract: A possible role for microglial CB_2 receptors in the etiology of schizophrenia is reviewed. CB_2 receptors are being expressed in the CNS, in contrary to earlier beliefs. These receptors are shown to play an important role on microglia, as modulators of neuroinflammation caused by microglia. Also, there is increasing evidence that neuroinflammation is involved in schizophrenia. Therefore, it seems that CB2 receptors are involved in the etiology of schizophrenia.

Introduction

Schizophrenia is a mental disorder that is defined for its symptoms in humans. As symptoms of schizophrenia affect a large number of cognitive functions that are considered to be exclusive to humans, it has been difficult to find suitable animal models to study what causes the disorder. Symptoms of schizophrenia are classified as either cognitive symptoms (such as working memory deficits), negative symptoms (which describe a wide range of inadequate social behavior) and positive symptoms (referring to psychotic symptoms such as hallucinations and delusions) (Stone *et al*., 2007). Nevertheless, some animal models have been described and verified to be suitable for studying some important mechanisms of schizophrenia (Belforte *et al.,* 2010). These animal models are based greatly on evidence that there are similarities between humans and animals in genetic factors and neurotransmitter system dysfunction that cause schizophrenia-like symptoms as they are classified above (Stone *et al*., 2007). Using these models, as well as other methods of research, dopaminergic-, glutamatergic-, GABAergic- and endocannabinoid neurotransmitter systems (Stone *et al*., 2007; Belforte *et al.,* 2010) and microglia (Monji *et al*., 2009) are demonstrated to be involved in the etiology of schizophrenia in both rats and humans. Since the discovery of cannabinoid receptors CB_1 and CB_2 in the early 1990s, researchers have been trying to find the mechanisms underlying the relationship between schizophrenia and the endocannabinoid system. Initial studies (Onaivi *et al*., 2006) showed that CB_1 receptors are expressed in the central nervous system (CNS), and CB_2 receptors are expressed peripherally. Because CB_2 receptors at that time were presumed to be absent in the brain, CB_1 receptors were thought to be the only cannabinoid receptors involved in the pathophysiology of schizophrenia. Further research indeed confirmed several links between schizophrenia and the cannabinoid CB_1 receptor; for example, upregulation of CB_1 receptors was reported in different prefrontal cortical areas in schizophrenic patients (Fernandez-Espejo *et al*., 2009). Also, certain CB_1 receptor polymorphisms were found to be associated with increased risk of developing schizophrenia (Fernandez-Espejo *et al*., 2009). Recent research, however, found evidence that CB_2 receptors too, are expressed in the CNS. Multiple studies found the expression of CB_2 receptors in microglia, as well as in several areas of the brain (Atwood & Mackie, 2010). Furthermore, Ishiguro *et al.* (2010) discovered that polymorphisms in the gene encoding for CB_2 receptors are also associated with schizophrenia, suggesting that CB_2 receptors might play a role in the etiology of schizophrenia. Currently, the best evidence for the presence of CB_2 receptors in the CNS is of their expression on microglia. Microglia fulfill several functions -such as the secretion of pro-inflammatory cytokines in response to neuroinflammation- and there is growing evidence that neuroinflammation is involved in schizophrenia (Monji *et al*., 2009). Therefore, CB_2 receptors might play their role in the etiology of schizophrenia through modulation of the activity of microglia. To examine this hypothesis, the expression and function of CB_2 receptors in the CNS will first be reviewed. Then, an attempt will be made to integrate knowledge of CNS CB_2 receptor location and function into the current theories on the mechanisms of schizophrenia.

Are CB_2 receptors expressed and functioning in the CNS?

Where are CB_2 receptors located in the CNS?

Before the discovery of CB_2 receptors in the CNS, CB_2 receptors were thought of as the 'peripheral' cannabinoid receptors because not only could CB_2 receptors not be identified in the brain, they also did not seem to be involved in the psychoactive effects of cannabinoids. CB_2 receptors were, however, found to be abundantly expressed in the immune system;

macrophages, CD4+ & CD8+ T cells, B cells, natural killer cells, neutrophils and monocytes, were all found to express CB_2 receptors. Furthermore, CB_2 receptors were identified in a number of different tissues, such as pulmonary endothelial cells, osteocytes, osteoclasts, osteoblasts, spleen, gastrointestinal system, reproductive system, retina and adipocytes (Atwood & Mackie, 2010). Further research showed that CB_2 receptors are inducible in reactive microglia, as a response to pathological conditions such as chronic neuroinflammation (Fernandez-Ruiz *et al*., 2008). Several studies went even further and found both direct and functional evidence for the expression of CB_2 receptors on microglia in normal circumstances, and even in multiple other substrates such as astrocytes, oligodendrocytes, hippocampal and cerebellar neurons (Onaivi *et al*., 2006; Brusco *et al*., 2008; Fernandez-Ruiz *et al*., 2008; Atwood & Mackie, 2010). These results were found using a number of different assays in humans, such as detection of CB_2 receptors and CB_2 receptor mRNA transcripts with immunohistochemistry, real time PCR and Western Blot analysis, and a far wider range of methods in animals (for a review of results acquired by these different assays, see Atwood & Mackie (2010) for both human and animal research, and Onaivi *et al*. (2006) for research in rats and mice). Also, it might be no coincidence that the studies that did not find CB_2 receptor expression in the CNS are generally older studies than the studies that do show CB_2 receptor expression in the CNS, as methods of detection keep improving (Atwood & Mackie, 2010). Even though the evidence for the expression of CB_2 receptors in these parts of the CNS is far from conclusive, most researchers seem to agree on two parts; CB_2 receptors are expressed on microglia, and are furthermore inducible, showing significant upregulation in response to pathological neuroinflammation.

How do microglial CB_2 receptors function?

It then becomes important as to why CB_2 receptors are induced or upregulated in microglia in response to neuroinflammation. Several studies (Onaivi *et al*., 2006; Fernandez-Ruiz *et al*., 2008; Atwood & Mackie, 2010) found evidence for multiple functions of CB_2 receptor activation in microglia; CB_2 receptor activation has been demonstrated to affect microglial proliferation, differentiation and migration, and maybe most notably, to decrease the production of neurotoxic substances by microglia. It has been well described that microglia, though normally beneficial for the CNS when activated as a result of short-term neural damage, may become a harmful factor during long-term or chronic neuroinflammation (Fernandez-Ruiz *et al*., 2008; Benito *et al*., 2008). This is because activated microglia produce and excrete substances that, as mentioned above, are neurotoxic. Some of these substances (such as nitric oxide, reactive oxygen species, IFN-γ and pro-inflammatory cytokines IL-1 β, IL-6 and TNF-α) can contribute to chronic neural damage, and CB_2 receptor activation has been shown to reduce microglial production of each of those substances in a number of different studies (Fernandez-Ruiz *et al*., 2008; Benito *et al*., 2008). Also, exogenous CB_2- specific agonists could be beneficial for treatment of several neurodegenerative pathologies, like Alzheimer's disease, HIV-induced encephalitis and multiple sclerosis. For instance, the CB_2- specific agonist JWH-015 seems to enhance removal of β-amyloid plaques in brain tissue sections of Alzheimer's disease patients, and to reduce microglial toxicity (Benito *et al.*, 2008). Thus, there is growing interest in the CB_2 receptor as potential target for new medicines that might stop, or even reverse, neurodegeneration. However, despite recent evidence that schizophrenia is at least partially a neurodegenerative disorder (Monji *et al.*, 2009), few to none studies have so far been published concerning the involvement of microglial expressed CB_2 receptors in the neuropathology of schizophrenia.

Are CB_2 receptors involved in the etiology of schizophrenia?

Dopamine and glutamate hypotheses of schizophrenia

Currently, there are several different hypotheses regarding what might be the etiology of schizophrenia. The discovery that the earliest antipsychotic drugs were all dopamine (DA) D_2 antagonists was the first evidence for neural dysfunction in schizophrenia. Thus, the first of the hypotheses of schizophrenia was the dopamine hypothesis of schizophrenia (Stone *et al*., 2007). In later studies, support was found for prefrontal hypofunction in schizophrenia, as well as evidence that this hypofunction is related to DA dysfunction in the prefrontal cortex, which could explain the cognitive symptoms of schizophrenia (Stone *et al*., 2007). Furthermore, DA hyperfunction

was found in the striatum, which is hypothesized to lead to positive symptoms of schizophrenia. Also, a polymorphism in the gene encoding COMT (catechol-O-methyltransferase -a DA metabolizing enzyme-) was found to be associated with schizophrenia (Stone *et al.*, 2007). However, as antipsychotics seemed to improve the positive symptoms, some pharmacological studies found that negative and cognitive symptoms are unaffected by DA D_2 antagonist antipsychotics (Stone *et al.*, 2007). In addition, the NMDA receptor antagonists ketamine and phencyclidine (PCP) were found to produce positive, as well as negative and cognitive schizophrenia-like symptoms. Therefore, in addition to the dopamine hypothesis, the NMDA hypofunction hypothesis of schizophrenia was proposed (Stone *et al.*, 2007). A recent study by Belforte *et al.* (2010) demonstrated that dysfunction of NMDA-receptors expressed on GABAergic inhibitory interneurons in prefrontal and hippocampal regions, leads to deficits in these interneurons in mice, and subsequently to disinhibition of glutamatergic excitatory neurons. This is in accordance with the hypothesis that glutamate hypofunction is one of the underlying causes of schizophrenic symptoms.

Microglia hypothesis of schizophrenia

Though it seems clear that DA and glutamate dysfunction are implicated in the etiology of schizophrenia, deficits in these neurotransmitter systems are very likely not the only cause of schizophrenia. This can be concluded from the discovery of several substrates and genetic or environmental factors that are associated with schizophrenia, but are not a part of DA or glutamate systems. These substrates and factors include the use of cannabis and genes important for the endocannabinoid system (Fernandez-Espejo *et al*., 2009; Ishiguro *et al.*, 2010), as well as microglia (Monji *et al.*, 2009). In a review by Stone *et al.* (2007), glutamate exitotoxicity was proposed as a possible cause for the observed deficit in GABAergic inhibitory interneurons. This might be the explanation for the observations that both grey matter volume is reduced in schizophrenia, and grey matter volume reduction precedes psychotic symptoms (Stone *et al.*, 2007), though further research has yet to prove whether this is indeed due to glutamate exitotoxicity, due to NMDA receptor dysfunction (as suggested by Belforte *et al.* (2010)) or even due to other causes. A recent meta-analysis by Monji *et al.* (2009), however, makes a more compelling case for the involvement of exitotoxicity or neuroinflammation in the etiology of schizophrenia. In this review, a large number of studies are highlighted that found either indirect or direct evidence for neurodegeneration, neuroinflammation and microglial activation in schizophrenia. For instance, increased serum concentrations of pro-inflammatory cytokines IL-2, IL-6 and IL-8 have been found in schizophrenia patients, as well as increased serum and cerebrospinal fluid concentrations of S100B; a marker for damage in the CNS (Monji *et al.*, 2009). Also, post-mortem studies suggested increased microglial density and activation in schizophrenia, and a positron emission computed tomography study found increased microglial activation in gray matter in schizophrenia patients, within the first 5 years after onset of schizophrenia (Monji *et al.*, 2009). This observation, along with very strong evidence that long-term microglial activation can induce apoptosis in neurons and oligodendrocytes by numerous mechanisms, makes microglial mediated apoptosis a plausible cause of the decrease in gray matter volume mentioned above (Monji *et al.*, 2009). These and other results have led to the proposal of a microglia hypothesis as another addition to the dopamine and glutamate hypotheses of schizophrenia (Monji *et al*., 2009).

Microglial CB_2 receptors and schizophrenia

Considering that microglia are indeed hyperactive in schizophrenia -and that activation of microglia leads to their migration to locations of neuroinflammation; to production and excretion of various neurotoxic substances; and ultimately to neural damage- modulation of the activation of microglia becomes an important issue. As mentioned above, there is evidence that the primary function of microglial CB_2 receptors is modulation of the neurotoxic substances that activated microglia produce and excrete. It thus seems likely that CB2 receptor dysfunction will result in an increased rate of microglial mediated neurodegeneration. A recent study demonstrated that CB_2 knock-out mice show symptoms such as increased anxiety and depression and impaired short-, and long-term memory function, which are in line with animal model negative and cognitive schizophrenic symptoms (Ortega-Alvaro *et al*., 2010). These symptoms were

furthermore reversible with the atypical antipsychotic risperidone. Another recent study found that the CB2 receptor reverse agonist AM630 worsens methamphetamine or MK-801 induced schizophrenic-model behavior (Ishiguro *et al.*, 2010). The same study, using post-mortem brain tissue from schizophrenic patients and controls, also described two functional polymorphisms in the gene encoding CB2 receptors that reduce CB2 receptor function. Both polymorphisms were found to be associated with increased risk of schizophrenia.

Discussion

Although research into the involvement of CB_2 functioning in the etiology of schizophrenia has only just begun, and several theoretical causations are yet to be experimentally proven, it seems likely that microglial CB_2 receptors do play an important role in limiting neuroinflammation that is caused by the microglia on which they are expressed. This hypothesis is supported by the findings that neurodegeneration is involved in the etiology of schizophrenia, that CB_2 receptors are highly inducible in microglia on which they have a neuroprotective role and that CB_2 receptor dysfunction is associated with schizophrenia. However, as Ishiguro *et al.* (2010) suggest, CB_2 receptor dysfunction is almost certainly not enough to develop schizophrenia. In the much the same way that long-term abuse of cannabis and subsequent change in CB_1 receptor activity is demonstrated to influence the dopamine and glutamate systems that are involved in the etiology of schizophrenia (Fernandez-Espejo *et al.*, 2009), chronic changes in CB_2 receptor activity and expression due to abuse of cannabis might add up as yet another factor that increases the risk of developing schizophrenia, though this has not yet been researched properly. This way, a fourth 'cannabinoid' hypothesis of schizophrenia can be integrated into the previously mentioned hypotheses as the sum of both CB_1 receptor, as CB_2 receptor mediated influence in the CNS. As CB_2 receptors have been confirmed to be expressed in the CNS (Onaivi *et al*., 2006) and shown to play an important role in neuroprotection when expressed on microglia, there are a number of factors concerning CB_2 receptors that have to be taken into account when interpreting results of recent studies. First of all, though evidence for CB_2 receptor expression in the CNS is strongest for expression on microglia, it is likely that future research will confirm CB_2 expression in other parts of the CNS as well. As mentioned earlier, astrocytes, oligodendrocytes, hippocampal and cerebellar neurons are all shown to express CB_2 receptors, so different mechanisms that are involved in the pathophysiology of schizophrenia might be mediated through CB_2-receptor expression and neural activity in those parts of the brain. Furthermore, CB_2 receptors are not the only receptors that are expressed on microglia. CB_1 receptors, among others, are also expressed on microglia, and much more abundant in the CNS. There might also be confounding from unintentional activation of these receptors, since a lot of ligands used in CB_2 receptor function assays also have some affinity for CB_1 receptors. Therefore, it is recommended that more recent and more selective ligands are used to replicate and validate previous results. Another very important problem in the effort of trying to understand and control the biology of schizophrenia are the developmental aspects of the etiology of the disease. These aspects are involved in nearly every factor that could influence the development of schizophrenia. For instance, schizophrenic symptoms usually develop in a particular order, with the negative and cognitive symptoms preceding the positive symptoms. The onset of the positive, psychotic symptoms is typically at the end of adolescence. Though Belforte *et al.* (2010) found that NMDA receptor NR1 subunit knockout mice showed schizophrenic symptoms, mice did not show schizophrenic symptoms when the gene encoding the NR1 subunit was not fully deleted until after adolescence. Also, multiple studies suggest that the greatest risk of developing schizophrenia due to the abuse of cannabis occurs during a certain window of development, in particular before late adolescence (Fernandez-Espejo *et al.*, 2009). As cannabinoid receptors are found on neural progenitor cells, and even stem cells, early influence by exogenous cannabinoids is prone to disturb natural development of the brain. These developmental aspects of schizophrenia and the influence of cannabinoids thereon are quite disadvantageous to the prospect of potential treatment of schizophrenia because they imply that when schizophrenia is diagnosed, the damage had been done and all that is left for the patient is relief of symptoms. Microglia and CB_2 receptors in the CNS may, however, be a useful target even further in the course of the disease to prevent additional neural damage. Other targets, such as

enzymes involved in degradation of (endo)cannabinoids or the endocannabinoid uptake carrier (that is yet to be characterized) may be subject of further research into the involvement of cannabinoids in the etiology of schizophrenia, or potential treatment of the disease.

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