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BRAIN RESEARCH

Research Report

The activation of cannabinoid CB_2 receptors stimulates *in situ* and *in vitro* beta-amyloid removal by human macrophages

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ARTICLE INFO

Article history:
Accepted 27 May 2009
Available online 6 June 2009

Keywords:
Cannabinoid
Amyloid
Macrophage
Cb2-receptor
Phagocytosis

ABSTRACT

The endocannabinoid system is a promising therapeutic target in a wide variety of diseases. However, the non-desirable psychotropic effects of natural and synthetic cannabinoids have largely counteracted their clinical usefulness. These effects are mostly mediated by cannabinoid receptors of the CB_1 type, that exhibit a wide distribution in neuronal elements of the CNS. Thus, the presence of other elements of this system in the CNS, such as CB_2 receptors, may open new possibilities for the development of cannabinoid-based therapies. These receptors are almost absent from the CNS in normal conditions but are up-regulated in glial cells under chronic neuroinflammatory stimuli, as has been described in Alzheimer's disease. To understand the functional role of these receptors, we tested their role in the process of beta-amyloid removal, that is currently considered as one of the most promising experimental approaches for the treatment of this disease. Our results show that a CB_2 agonist (JWH-015) is capable of inducing the removal of native beta-amyloid removal from human frozen tissue sections as well as of synthetic pathogenic peptide by a human macrophage cell line (THP-1). Remarkably, this effect was achieved at low doses (maximum effect at 10 nM) and was specific for this type of cells, as U373MG astrocytoma cells did not respond to the treatment. The effect was CB_2-mediated, at least partially, as the selective CB_2 antagonist SR144528 prevented the JWH-015-induced plaque removal *in situ*. These data corroborate the possible therapeutic interest of CB_2 cannabinoid specific chemicals in the treatment of Alzheimer's disease.

1. Introduction

Cannabinoids have been used during centuries for the treatment of different diseases (for a review, see Mechoulam and Hanus, 2000). Until recently, the molecular basis of the therapeutic and psychoactive effects of these compounds were unknown (Howlett et al., 2002). The pharmacological characterization and subsequent cloning of a specific membrane receptor (the CB_1 receptor) identified the target of these compounds and gave support to the search for its endogenous

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doi:10.1016/j.brainres.2009.05.098

ligand(s). These ligands were identified as a family of lipid-related molecules that are released "on demand" from precursors present in the cell membrane. Among these so called "endocannabinoids", the ethanolamine of the arachidonic acid ("anandamide", AEA) and the 2-arachydonoylglycerol (2-AG) are most relevant. Shortly after, other elements of the endocannabinoid system (ECS) were also described. Thus, the molecular machinery for the synthesis and degradation of the endocannabinoids is now known to include several routes for their synthesis, a partially characterized uptake mechanism (Kaczocha et al., 2009) and degradative enzymes (such as the fatty acid amide hydrolase and the monoglycerol lipase) (Howlett et al., 2002).

It is also currently accepted that natural, synthetic and endogenous cannabinoids bind and activate other types of receptors. Remarkably, a second cannabinoid receptor was identified in 1993 and termed CB_2 (Munro et al., 1993). In contrast with CB_1 (present in several organs and tissues, with an outstanding abundance in CNS neurons), CB_2 receptors were initially located only in peripheral elements of the immune system (Lynn and Herkenham, 1994). Until very recently, CB_2 receptors were thought to be absent from the CNS. However, seminal studies from Cabral's group suggested that CB_2 receptors have an inducible nature (reviewed in Cabral and Marciano-Cabral, 2005). These authors found that the expression of this receptor varies as a function of the cellular activational state in several murine cell lines (Carlisle et al., 2002). The actual expression level of CB_2 receptors in normal conditions is still under intense debate (Fernández-Ruiz et al., 2007; Zhang et al., 2008). Afterwards, several *in vitro* and *in vivo* studies have revealed that CB_2 receptors are up-regulated after chronic pro-inflammatory stimuli (for a review, see Benito et al., 2007b). Recent data obtained from human tissue samples indicate that the expression of CB_2 receptors in the CNS is dramatically increased in several chronic neurodegenerative diseases, including multiple sclerosis (Benito et al., 2007a), viral encephalitis (Benito et al., 2005), Down's syndrome (Núñez et al., 2008) and Alzheimer's disease (AD; Benito et al., 2003). In these diseases, CB_2 receptors seem to be selectively overexpressed in glial cells and, remarkably, in microglial cells and infiltrated macrophages.

On the other hand, it is currently accepted that the deposition of the beta-amyloid-(1-42)-peptide (Aβ) is one of the main features of AD and seems to trigger a cascade of neuroinflammatory events that ultimately leads to neurodegeneration (McGeer et al., 2000; Walsh and Selkoe, 2004). AD affects more than 25 million people worldwide and its incidence is expected to grow with the increase in life expectancy in developed countries. Furthermore, effective treatments for this disease are lacking as only palliative approaches are currently available (Lleó et al., 2006). Among them, acetylcholinesterase inhibitors (donepezil, rivastigmine, etc.) and antagonists for the N-methyl-D-aspartate glutamatergic receptors (memantine) are used in current clinical practice (Lleó et al., 2006). It has also been recently proposed that decreasing Aβ burden through the use of specific vaccines may constitute a novel and promising therapeutic approach (for review, see Wyss-Coray, 2006).

The role of neuroinflammation and microgliosis in AD is attracting considerable attention as recent data suggest that both factors are early events in the pathogenesis of this disease (Kitazawa et al., 2005; Wyss-Coray, 2006; Yoshiyama et al., 2007). Thus, the present study was designed to explore if CB_2 up-regulation in Aβ-associated cells of myeloid origin may contribute to the role that these cells play in Aβ removal. To that end, we tested the effects of the CB_2 agonist JWH-015 on the activity of two different human cell lines, each of them representative of the two main types of glial cells involved in neuroinflammation: U373MG, as model of astrocytes, and THP-1 derived macrophages, a widely used model of microglia (Koenigsknecht and Landreth, 2004). We analyzed their ability to remove two different species of Aβ: i) Aβ-enriched neuritic plaques from frozen human tissue sections; and ii) fibrillar assemblies of synthetic Aβ.

2. Results

In concordance with the clinical and neuropathological diagnosis, frozen temporal cortex tissue sections exhibited high levels of Aβ immunoreactivity (Fig. 1). On the other hand, and confirming previous reports from other groups (Aguado et al., 2007; Klegeris et al., 2003), both U373MG and THP-1 cell lines were shown to express CB_2 receptors, as revealed by the presence of measurable mRNA levels by quantitative RT-PCR (data not shown). This observation corroborates previous reports on the expression of CB_2 receptors in THP-1 (Rajesh et al., 2008) and U373MG cells (Aguado et al., 2007).

U373 astrocytoma cells were not able of removing significant amounts of Aβ plaques either alone or when treated with any of the concentrations of the specific CB_2 agonist (Fig. 1). THP-1 macrophages also showed no ability to remove pathological deposits by themselves or when exposed to vehicle (DMSO) (Fig. 1). However, when incubated with very low levels of JWH-015 (1 nM), plaque burden was dramatically reduced (by 39±13%). Higher concentrations of the CB_2 agonist were also effective, reaching a maximum effect at 5 nM and 10 nM (decreases of 64±9% and 65±8%, respectively). Treatment with 100 nM JWH-015 triggered a lower response, with decreased ability to remove Aβ plaques by THP-1 cells (decrease of 28±4% in relative plaque area) (Fig. 1). The concentration of 10 nM was chosen for further experiments, as it induced the maximal effect observed.

JWH-015 has been shown to bind CB_2 receptors with higher selectivity (K_i 13.8 nM) over CB_1 receptors (K_i 383 nM) (Howlett et al., 2002). In addition, the low concentrations used in this study suggest a CB_2-mediated action of this compound. In order to corroborate a CB_2 mediation of the observed effect, the selective CB_2 antagonist SR144528 was employed. Thus, cells were co-incubated with JWH-015 at the most effective concentration (10 nM) and SR144528 (25 nM) and their ability to remove pathological deposits measured. By this procedure, prevention by the treatment with the CB_2 antagonist could be observed (Fig. 2).

We then sought to confirm these data by using synthetic Aβ. *In vitro* experiments confirmed the stimulatory effect of JWH-015 on the phagocytosis of Aβ by THP-1 macrophages (Fig. 3). Thus, fluorimetric measurement of Cy3-labelled Aβ indicated that JWH-015 (10 nM)-stimulated cells removed significantly higher amounts of the peptide, with decreases of