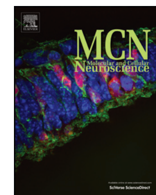
Molecular and Cellular Neuroscience xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne



Role of the cannabinoid system in the transit of beta-amyloid across the blood–brain barrier

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ARTICLE INFO

Article history:
Received 26 March 2013
Revised 30 May 2013
Accepted 25 June 2013
Available online xxxx

Keywords:
Alzheimer's disease
Amyloid
Cannabinoid
Blood–brain barrier
Clearance
Low density lipoprotein receptor-related protein 1

ABSTRACT

Emerging evidence suggests beta-amyloid (Aβ) deposition in the Alzheimer's disease (AD) brain is the result of impaired clearance, due in part to diminished Aβ transport across the blood–brain barrier (BBB). Recently, modulation of the cannabinoid system was shown to reduce Aβ brain levels and improve cognitive behavior in AD animal models. The purpose of the current studies was to investigate the role of the cannabinoid system in the clearance of Aβ across the BBB. Using *in vitro* and *in vivo* models of BBB clearance, Aβ transit across the BBB was examined in the presence of cannabinoid receptor agonists and inhibitors. In addition, expression levels of the Aβ transport protein, lipoprotein receptor-related protein1 (LRP1), were determined in the brain and plasma of mice following cannabinoid treatment. Cannabinoid receptor agonism or inhibition of endocannabinoid-degrading enzymes significantly enhanced Aβ clearance across the BBB (2-fold). Moreover, cannabinoid receptor inhibition negated the stimulatory influence of cannabinoid treatment on Aβ BBB clearance. Additionally, LRP1 levels in the brain and plasma were elevated following cannabinoid treatment (1.5-fold), providing rationale for the observed increase in Aβ transit from the brain to the periphery. The current studies demonstrate, for the first time, a role for the cannabinoid system in the transit of Aβ across the BBB. These findings provide insight into the mechanism by which cannabinoid treatment reduces Aβ burden in the AD brain and offer additional evidence on the utility of this pathway as a treatment for AD.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative process characterized by neuronal cell loss (Donev et al., 2009) and a global decline in cognitive function (Citron, 2010). The major pathological hallmarks of AD include the formation of neurofibrillary tangles and the deposition of beta-amyloid proteins (Aβ) in the brain and cerebrovasculature (Mehta, 2007). Moreover, AD pathophysiology is associated with increased neuroinflammation and oxidative stress (Galasko and Montine, 2010) in addition to excitotoxicity and dysregulated calcium homeostasis (LaFerla, 2002). While the etiology and pathogenesis of AD are poorly understood, Aβ accumulation in the brain appears to be a key factor in the development of AD as it precedes the neuroinflammatory and neurotoxic aspects of this disease (LaFerla et al., 2007). Furthermore, soluble Aβ levels in the brain correlate with the severity of neurodegeneration (McLean et al., 1999; Weller et al., 2009) and are a predictor of cognitive impairment in AD (Nordberg, 2008).

An increasing body of evidence suggests a neuroprotective role for the cannabinoid system in the brain that may have applications in the treatment of neurodegenerative disorders, including AD (Campbell and Gowran, 2007; Sarne and Mechoulam, 2005). The cannabinoid system consists of two G protein-coupled membrane receptors (CB1 and CB2) and a number of endogenous agonists (endocannabinoids), one of which is 2-arachidonoyl-glycerol (2-AG) (Kirilly et al., 2012). 2-AG activity is predominantly regulated by monoacylglycerol lipase (MAGL) (Dinh et al., 2002), and to a lesser extent, fatty acid amide hydrolase (FAAH) (Bisogno et al., 2002) and α/β-hydrolase domain 6 (ABHD6) (Marrs et al., 2010). These enzymes hydrolyze and inactivate 2-AG and, as a consequence, mitigate signaling through the CB receptors (Savinainen et al., 2012). In addition, hydrolysis of 2-AG in the brain generates arachidonic acid and the production of neuroinflammatory prostaglandins. Disruption of MAGL activity suppresses these inflammatory processes, resulting in neuroprotection (Nomura et al., 2011). The neuroprotective function of the cannabinoid system is thought to occur through a variety of mechanisms. Activation of CB1, which controls the release of excitatory neurotransmitters from the pre-synaptic neuron, can protect against excitotoxicity (Marsicano et al., 2003; Shen and Thayer, 1998) and promote neurogenesis (Aguado et al., 2007; Jiang et al., 2005; Jin et al., 2004). Meanwhile, stimulation of the CB2 receptor attenuates oxidative stress (Horvath et al., 2012) and reduces neuroinflammation by suppressing microglial activation (Ehrhart et al., 2005) and controlling the production of inflammatory mediators (Campbell and Gowran, 2007).

With respect to the cannabinoid system and AD, a number of recent studies explored the influence of the cannabinoid system on Aβ accumulation in the brain. CB receptor stimulation reduced Aβ

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1044-7431/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.mcn.2013.06.004

Please cite this article as: Bachmeier, C., et al., Role of the cannabinoid system in the transit of beta-amyloid across the blood–brain barrier, Mol. Cell. Neurosci. (2013), http://dx.doi.org/10.1016/j.mcn.2013.06.004

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brain deposition (Martin-Moreno et al., 2012; Wu et al., 2013), restored synaptic plasticity, and improved cognitive behavior in mouse models of AD (Aso et al., 2012; Haghani et al., 2012). Similarly, inactivation of the MAGL enzyme lowered Aβ brain burden (Chen et al., 2012; Piro et al., 2012) and improved long-term synaptic plasticity, spatial learning, and memory (Chen et al., 2012; Pan et al., 2011) in transgenic AD animals. Recent work has suggested the impact of the cannabinoid system in ameliorating Aβ brain burden is the result of an increased transport of Aβ out of the brain (Martin-Moreno et al., 2012). As Aβ transit across the blood–brain barrier (BBB) is an important determinate of Aβ accumulation in the AD brain, we investigated the role of the cannabinoid system in the clearance of Aβ across the BBB. We found that endocannabinoid treatment or MAGL inhibition facilitated Aβ transit across the BBB *in vitro* and *in vivo*. This effect appears to be the result of increased expression of the low density lipoprotein receptor-related protein 1 (LRP1), which is known to participate in the brain-to-blood transport of Aβ (Shibata et al., 2000). These studies suggest a role for the cannabinoid system in the elimination of Aβ from the brain to the periphery and may explain the impact of this system on Aβ brain burden and AD pathophysiology.

Results

Cannabinoid receptor agonism

The role of the cannabinoid pathway in fluorescein-Aβ(1-42) transcytosis was examined using an established *in vitro* model of the BBB (Bachmeier et al., 2010). Cannabinoid receptor stimulation with an endocannabinoid agonist (2-AG) dose-dependently enhanced the basolateral-to-apical transit of Aβ across the BBB model, increasing Aβ transcytosis to nearly twice that of control (Fig. 2). The synthetic CB receptor agonist, CB13, also facilitated Aβ transcytosis across the BBB model, though the impact of this compound was not as potent or as pronounced as 2-AG (Fig. 2). Aβ BBB transcytosis was also significantly altered upon treatment with the MAGL inhibitors JZL184 and JZL195. The profile with these drugs was similar to that observed for 2-AG, resulting in an approximately 2-fold increase in Aβ BBB transit compared to control (Fig. 3A). It should be noted that JZL195 is a dual inhibitor of MAGL and FAAH (Long et al., 2009b), whereas JZL184 is selective for MAGL only (Long et al., 2009a). We also examined the impact of other 2-AG hydrolyzing enzymes (FAAH and ABHD6) in the BBB model. Treatment with the FAAH inhibitors JNJ166 and PF570 had no effect on Aβ transit at concentrations up to 10 μM (Fig. 3B). Similar results were obtained for the ABHD6 enzyme inhibitor, WWL70 (Fig. 3C). While these drugs did demonstrate an effect at 20 μM, the magnitude of this effect was less than that observed with MAGL inhibition or direct 2-AG agonism.

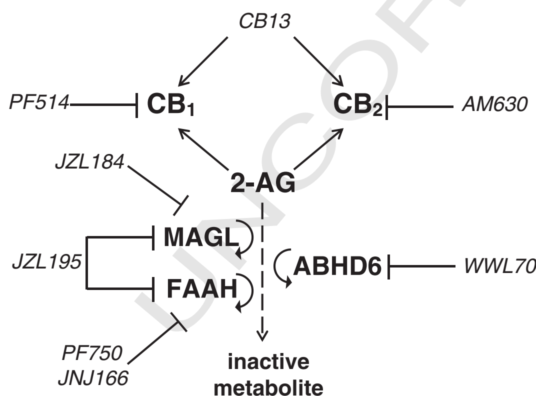

Fig. 1. Schematic of the cannabinoid pathway and the modulators used in the current studies. The cannabinoid receptors, CB1 and CB2, are both stimulated by 2-AG (endogenous agonist) and CB13 (synthetic agonist) and selectively inhibited by PF514 (CB1) and AM630 (CB2). 2-AG is hydrolyzed by the enzymes MAGL, FAAH, and ABHD6, which inactivates 2-AG and mitigates CB receptor stimulation. Alternatively, inhibitors of MAGL (JZL184, JZL195), FAAH (PF750, JNJ166), and ABHD6 (WWL70) attenuate 2-AG conversion, which promotes stimulation of the CB receptors. Note: JZL195 is an inhibitor of both MAGL and FAAH. Straight arrows indicate stimulation, blunt lines indicate inhibition, curved arrows indicate metabolism, and the dotted line indicates conversion to an inactive metabolite. For illustrative purposes, not all of the molecules that influence the cannabinoid pathway are depicted.

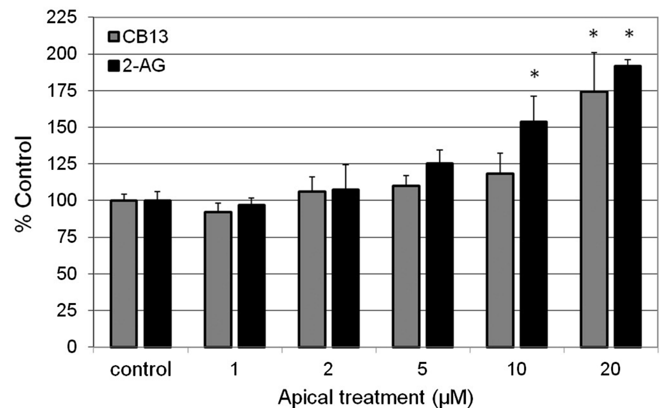

Fig. 2. Basolateral-to-apical transcytosis of fluorescein-Aβ(1-42) across an *in vitro* model of the BBB in the presence of an endogenous (2-AG) or synthetic (CB13) cannabinoid agonist. Fluorescein-Aβ(1-42) (2 μM) was exposed to the basolateral ("brain") compartment while various concentrations of each agonist (1, 2, 5, 10, and 20 μM) were exposed to the apical ("blood") compartment of the BBB model. Samples were collected from the apical compartment at 60 min to determine fluorescein-Aβ(1-42) transcytosis across the BBB model and the values expressed as a percentage of control. Values represent mean ± SEM (n = 3). *P < 0.05 compared to control as determined by ANOVA and Bonferroni post-hoc test.

Cannabinoid receptor modulation

To determine whether the effects we observed with endocannabinoid agonism and MAGL inhibition were mediated through the CB receptors, we examined Aβ BBB transcytosis upon direct CB receptor inhibition. Aβ BBB transcytosis was unaffected by CB receptor modulation as treatment with inhibitors of the CB1 (PF514) and/or CB2 (AM630) receptors did not alter the basal rate of Aβ transit across the BBB model (Figs. 4 and 5). However, when the CB receptors were stimulated with 2-AG or the MAGL inhibitor, JZL195, we observed a reduction in Aβ BBB transcytosis in the presence of CB receptor antagonism. For CB1 inhibition, we observed approximately a 50% reduction in 2-AG or JZL195 stimulated Aβ BBB transit for each concentration of PF514 tested (Fig. 4). CB2 inhibition by AM630 resulted in a dose-dependent decrease in Aβ BBB transit following receptor stimulation by 2-AG or JZL195 (Fig. 5). At 10 μM, AM630 completely negated the stimulatory effects of 2-AG and JZL195, reducing Aβ BBB transcytosis to control levels (Fig. 5). Interestingly, when both CB1 and CB2 were inhibited simultaneously, not only were the stimulatory effects of 2-AG and JZL195 fully negated, but Aβ BBB transcytosis was diminished below baseline to approximately half that observed under control conditions (Fig. 6). Alternatively, we also examined the effect of cannabinoid receptor modulation on the brain entry of Aβ (*i.e.*, "blood"-to-"brain" direction). Once again, CB receptor inhibition alone did not influence Aβ BBB transit, however, CB receptor stimulation with 2-AG significantly reduced (>40%) the BBB penetration of Aβ in the *in vitro* model (Fig. 7). When the treatments were combined, CB receptor blockade slightly abrogated the stimulatory effects of 2-AG on Aβ BBB entry, nevertheless Aβ transit remained well below that observed for control (Fig. 7).

Aβ BBB clearance in vivo

Using an *in vivo* model of Aβ clearance across the BBB (Paris et al., 2011), we intracranially administered human Aβ(1-42) to wild-type mice and examined the appearance of Aβ(1-42) in the plasma

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