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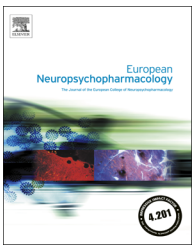

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In vivo type 1 cannabinoid receptor availability in Alzheimer's disease

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Abstract
The endocannabinoid system (ECS) is an important modulatory and potentially neuroprotective homeostatic system in the brain. In Alzheimer's disease (AD), the role of type 1 cannabinoid receptor (CB_1R) is unclear, with contradictory findings in post-mortem studies showing upregulation, down-regulation or unchanged CB_1R status. We have investigated CB_1R availability *in vivo* in patients with AD, in relation to amyloid deposition, cognitive functioning and apolipoprotein E (ApoE) genotype. Eleven AD patients and 7 healthy volunteers (HV) underwent combined [^{18}F]MK-9470 PET and [^{11}C]PIB PET scans to assess CB_1R availability and amyloid deposition, respectively, and T1 volumetric MRI for partial volume correction. We found no difference in CB_1R availability between AD and HV, VOI-based fractional uptake values (FUR) were 0.043 ± 0.01 for AD and 0.045 ± 0.01 for controls ($p=0.9$). CB_1R availability did not correlate with neuropsychological test scores and was not modulated by ApoE genotype. As expected, global [^{11}C]PIB SUVR (standardized uptake value ratio) was increased in AD (SUVR 1.9 ± 0.3) compared to HV (1.2 ± 0.1) with $p<0.001$, but no correlation was found between amyloid β (Aβ) deposition and CB_1R availability. In conclusion, we found no *in vivo* evidence for a difference in CB_1R availability in AD compared to age-matched controls. Taken together with recently reported *in vivo* CB_1R changes in Parkinson's and Huntington's disease, these data suggest that the CB_1R is differentially involved in neurodegenerative disorders.

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1. Introduction

Impairment of the cholinergic system and its relation to cognitive dysfunction is well-known in Alzheimer's disease (AD). Various other neurotransmitter systems (e.g. the serotonergic system (Rodriguez et al., 2012)) and concomitant changes of associated receptors and synthetic enzymes have been related to cognitive and behavioral changes. Recent advances in the characterization of different functions of the endocannabinoid system (ECS) suggest that this neurotransmitter system may also play a role in the pathophysiology of AD in different ways as well as in the pathogenesis of cognitive dysfunction. The ECS is generally viewed as a neuromodulatory system that interacts with, and regulates several neurotransmitter systems (Terranova et al., 1996). Growing evidence also shows that type 1 cannabinoid receptors (CB_1R) play a fundamental role in neuroprotection including in AD (Aso et al., 2012). *In vitro* experiments suggest that endogenous cannabinoids promote changes in neural activity related to memory, with a role in long-term plasticity (Ramirez et al., 2005). Overall, *in vivo* experiments with mice have been ambiguous, with reports of both impaired and enhanced memory performance (Ledent et al., 1999; Reibaud et al., 1999). Administration of CB_1R antagonists improved memory in a rodent model of AD, probably through modulation of acetylcholine (Ach) levels (Davies et al., 2002). On the other hand, it has also been shown that chronic administration of the CB_1R agonist arachidonyl-2-chloroethylamide (ACEA) reduces cognitive impairment observed in double AβPP(swe)/PS1(1dE9) transgenic mice probably through GSK3β inhibition, reduction of reactive astrocytes and lower expression of interferon-γ (Aso et al., 2012). CB_1Rs are also involved in mediating the Aβ neurotoxicity and in protecting against amnesia in hippocampal learning tasks. SR141716A, a CB_1R antagonist, improves amnesia induced by Aβ fragments in mice, suggesting that endogenous cannabinoids may be involved in cognitive impairment induced by these fragments (Mazzola et al., 2003). In humans, Walther et al. showed significant improvement of the Neuropsychiatric Inventory scores in late onset dementia after a daily administration of dronabinol, a cannabinoid agonist (Walther et al., 2006).

Post-mortem studies in AD on the role of the CB_1R and its relation to cognitive function at end-of-life remain unclear. Ramirez et al. reported loss of CB_1R – positive neurons in the frontal cortex of AD patients, decreased CB_1 protein expression and G-protein decoupling, despite preserved density and binding of the receptor (Ramirez et al., 2005). They also showed consistent CB_1R immunoreactivity in senile plaques along with markers of microglial activation, suggesting a direct involvement of these receptors in the effects of microglia. In contrast, Westlake et al. found reduced CB_1R density in several areas including the entorhinal cortex and hippocampus, but no association between reduced CB_1R expression and neuropathological signs of AD (Westlake et al., 1994). Benito et al. found no changes in CB_1R density in the proximity of neuritic plaques (Benito et al., 2003) and also other recent studies described preserved expression of CB_1R, even in severe AD (Farkas et al., 2012; Lee et al., 2010).

Over the past years, several positron emission tomography (PET) radioligands have been developed that allow *in vivo* quantification of the CB_1R distribution, such as [^{18}F]MK-9470 (Burns et al., 2007; Sanabria-Bohorquez et al., 2010), [^{11}C]OMAR (Horti et al., 2006) and [^{18}F]MPePP (Terry et al., 2008). The aim of this study was to measure the *in vivo* CB1R status in AD in relation to Aβ deposition, cognitive parameters and apolipoprotein E (ApoE) genotype. We therefore conducted a prospective, cross-sectional multitracer study using [^{18}F]MK-9470 and [^{11}C]PIB in mild to moderate AD patients and healthy controls.

2. Experimental procedure

2.1. Subjects

AD patients had to meet following inclusion criteria: (1) $\geq$ 55 years of age; (2) diagnosis of probable AD according to the NINCDS-ADRDA Criteria (Dubois et al., 2007); (3) magnetic resonance imaging (MRI) scan obtained within the last 12 months consistent with a diagnosis of AD; (4) Modified Hachinski Ischemic Scale (MHIS) score of $\leq$ 4; (5) global CDR (Clinical Dementia Rating score) (Morris, 1993) between 1 and 3, or, if the Global CDR is 0.5, then CDR Sum of Boxes of at least 3.5; (6) at least six years of education, or work history sufficient to exclude mental retardation, and (7) a positive [^{11}C]PIB PET scan. Thirteen patients with probable AD (5 men (M), 8 women (F); age range 57.6–81.8 years) were screened. Two male patients fulfilling screening criteria 1–6 but with a negative [^{11}C]PIB PET scan, were excluded from the study. The patient group therefore consisted of 3 M and 8 F patients (age range 57.6–80.9 years). AD patients were compared to a group of 7 healthy cognitive intact and age-matched elderly volunteers (3 men, 4 women; age range 61.3–79.0 years). These volunteers were prospectively recruited in response to advertisements in local community newspapers and departmental website.

2.2. Neuropsychological evaluation

All subjects underwent thorough neuropsychological evaluation. The following tests were conducted: Dutch version of the mini-mental state examination (MMSE) (O'Bryant et al., 2008), auditory verbal learning test (AVLT) (Balthazar et al., 2010; Van der Elst et al., 2005), Boston naming test (BNT) (Karrasch et al.), Raven's colored progressive matrices test (RCPMT), the subtest Object Decision (OD) of the visual object and space perception test (VOSP) (Videaud et al., 2008), clinical dementia rating (CDR) scale (Morris, 1993), the cognitive part of the Alzheimer's disease assessment scale (ADAS-cog) (Skinner et al., 2012), neuropsychiatric inventory (NPI) (Cummings, 1997), Alzheimer's disease cooperative study – activities of daily living (ADCS-ADL) (Galasko et al., 1997), and geriatric depression scale (GDS) (Albinski et al., 2011). Only MMSE and AVLT (total learning (A1–A5), delayed and recognition scores of the AVLT), measures of global cognitive functioning and episodic memory respectively, were used for correlation analyses with CB_1R availability. CDR sum of boxes was used as a variable of interest in the correlation analysis with AVLT scores. We have also added the maximum scores to Table 1. Blood sampling for ε4 allele(s) of ApoE ε4 was done in all except one subject that died shortly after the PET scans. The study was approved by the local Ethics Committee and performed in accordance to the latest version of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from healthy controls, subjects with AD and from their primary caregivers, prior to the study.

2.3. Radiotracer characteristics and preparation

The [^{18}F]MK-9470 precursor was obtained from Merck Research Laboratories and labeled at the PET site using ^{18}F-ethylbromide