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[^{18}F]FMPEP-d_2 PET imaging shows age- and genotype-dependent impairments in the availability of cannabinoid receptor 1 in a mouse model of Alzheimer's disease

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ABSTRACT

Contradictory findings on the role of the type 1 cannabinoid receptor (CB_1R) during the pathogenesis of Alzheimer's disease (AD) have been reported. Here, we evaluated the CB_1R brain profile in an AD mouse model using longitudinal positron emission tomography with an inverse agonist for CB_1R, [^{18}F]FMPEP-d_2. APP/PS1-21 and wild-type (n = 8 in each group) mice were repeatedly imaged between 6 to 15 months of age, accompanied by brain autoradiography, western blot, and CB_1R immunohistochemistry with additional mice. [^{18}F]FMPEP-d_2 positron emission tomography demonstrated lower ($p < 0.05$) binding ratios in the parietotemporal cortex and hippocampus of APP/PS1-21 mice compared with age-matched wild-type mice. Western blot demonstrated no differences between APP/PS1-21 and wild-type mice in the CB_1R abundance, whereas significantly lower ($p < 0.05$) receptor expression was observed in male than female mice. The results provide the first demonstration that [^{18}F]FMPEP-d_2 is a promising imaging tool for AD research in terms of CB_1R availability, but not expression. This finding may further facilitate the development of novel therapeutic approaches based on endocannabinoid regulation.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive memory loss, cognitive decline, and the accumulation of neuritic β-amyloid plaques and neurofibrillary tangles, associated with elevated neuroinflammation and oxidative stress (Ball, 1976; Glenner et al., 1984). Strong evidence of alterations in the endocannabinoid system (ECS) in the pathogenesis of AD has raised questions about the development of novel therapeutic approaches for AD based on endocannabinoid regulation (Fagan and Campbell, 2015). The ECS is composed of a relatively broad set of receptors, endogenous ligands, and enzymes, which are involved in AD pathogenesis (Karl et al., 2012; Pazos et al., 2004). The type 2 cannabinoid receptor is overexpressed in activated microglia (Benito et al., 2003); however, the role of the type 1 cannabinoid receptor (CB_1R) is unclear because contradictory results from postmortem human AD studies show bipolar changes in receptor regulation or unchanged CB_1R status (Ahmad et al., 2014; Lee et al., 2010; Ramirez et al., 2005; Westlake et al., 1994). Preclinical studies with AD animal models have also yielded contradictory results. Reductions in amyloid plaque load accompanied with impaired learning and memory deficits were demonstrated in CB_1R-deficient APP23 mice (APP23/CB1$^{-/-}$) when compared with APP23 mice, suggesting that CB_1R deficiency worsens learning and memory deficits in AD

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(Stumm et al., 2013). Significantly decreased CB_1R expression has been observed in the hippocampus (HIPPO) of 10- to 12-month-old $APP_{SWE}/PS1_{\Delta E9}$ mice in association with astrogliosis (Kalifa et al., 2011), whereas increased CB_1R levels have been reported in the cortex of 14-month-old—but not in 7-month-old—$APP_{SWE}/PS1_{\Delta E9}$ mice (Mulder et al., 2011). Moreover, a recent study demonstrated no differences in CB_1R activity in 13- to 14-month-old female $APP_{SWE}/PS1_{\Delta E9}$ mice compared with wild-type (WT) mice (Karkkainen et al., 2012), whereas in 3×Tg-AD mice, thalamic CB_1R activity was up-regulated at 4 months of age, but not in older animals (Manuel et al., 2016).

Consequently, novel CB_1R positron emission tomography (PET) radioligands have been developed to monitor this receptor in CB_1R-related neuronal diseases in vivo. The first CB_1R-related radioligand, (−)-5′-[^{18}F]fluoro-Δ^8-THC, possessed high nonspecific binding, poor blood-brain barrier permeability, and low affinity for the target receptor (Charalambous et al., 1991). It was followed by many more "first-generation" radioligands, such as [^{18}F]AM5144 and [^{11}C]SR149080 (Li et al., 2005; Mathews et al., 2000). The initial "second-generation" radioligand, [^{11}C]OMAR, a rimonabant-like CB_1R antagonist, was reported to have reduced lipophilicity and higher affinity (Horti et al., 2006). Another second-generation radioligand, [^{18}F]MK-9470, is a potent CB_1R inverse agonist with a high affinity, 60-fold selectivity for CB_1R over type 2 cannabinoid receptor, and high specific binding to mammalian brain (Burns et al., 2007).

The newest member of the second-generation CB_1R-PET family is a structural analog for [^{11}C]MePPEP, [^{18}F]FMPEP-d_2, which is extremely lipophilic ($logD_{7.4} \approx 4.2$), yet has >80% specific binding in rhesus monkey brain and is less prone to in vivo defluorination due to its dideuterofluoromethoxy group (Terry, 2009; Tsujikawa et al., 2014). [^{18}F]FMPEP-d_2 has been used in a PET study for imaging brown adipose tissue (Eriksson et al., 2015) and detecting the involvement of the CB_1R system in alcohol dependence (Hirvonen et al., 2013). In AD research, the applicability of this tracer has yet to be examined.

Despite the difficulties of developing suitable PET radioligands for imaging CB_1Rs in vivo, the need is urgent for reliable research tools for studying the role of the ECS in neurological diseases. Therefore, the aim of this study was to evaluate the changes in CB_1Rs and the alterations in [^{18}F]FMPEP-d_2 binding in an aging transgenic (TG) mouse model of AD, APP/PS1-21, using longitudinal [^{18}F]FMPEP-d_2 PET/computed tomography (CT) imaging, ex vivo digital autoradiography, and western blot. Thin-layer chromatography and receptor blocking were used for investigating the metabolism and specific binding of [^{18}F]FMPEP-d_2, respectively. CB_1R abundance in mouse brain was visualized using immunohistochemistry. We hypothesize that [^{18}F]FMPEP-d_2 PET imaging can be used to image the CB_1R-associated pathogenesis in AD, thus paving the way for expanding future drug development strategies.

2. Materials and methods

2.1. [^{18}F]FMPEP-d_2 synthesis

[^{18}F]FMPEP-d_2 ([3*R*,5*R*]-5-((3-([^{18}F]fluoromethoxy-d_2)phenyl)-3-((*R*)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one) was synthesized at the Radiopharmaceutical Chemistry Laboratory at the Turku PET Centre. Precursor (3*R*,5*R*)-5-(3-hydroxyphenyl)-3-((*R*)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one was supplied by the commercial supplier (PharmaSynth, Tartu, Estonia), and [^{18}F]FMPEP-d_2 syntheses were conducted as described previously (Donohue et al., 2008). For radionuclide production, fluorine-18 was produced by proton irradiation of oxygen-18. [^{18}F]FMPEP-d_2 was formulated in up to 10% ethanol in saline. The molar activity was >500 GBq/μmol at the end of each synthesis, and the molar activity at the time of the injection was calculated according to the limiting value (500 GBq/μmol) with an average of 359 GBq/μmol (standard deviation [SD] = 71 GBq/μmol) (34 batches). The radiochemical purity exceeded 95% in all syntheses.

2.2. Experimental animals

APP/PS1-21 TG mice (C57BL/6J−TgN(Thy1−APPKM670/671NL; Thy1−PS1L166P) were originally purchased from Koesler (Rottenburg, Germany) and further bred with C57BL/6Cn mice in the Central Animal Laboratory of University of Turku. APP/PS1-21 contains human transgenes for both amyloid precursor protein bearing the Swedish mutation and presenilin 1 containing L166P mutation, both under the control of the Thy1 promoter (Radde et al., 2006). Toxic Aβ42 begins to develop in the brain of this mouse model at 6 weeks of age, and peak number of the fibrillary deposits is reached at 9 months of age (Takkinen et al., 2017). WT mice from the same litter were used as control animals. All animals were housed and fed as described previously (Takkinen et al., 2017). All animal experiments were approved by the Regional State Administrative Agency for Southern Finland (ESAVI/3899/04.10.07/2013), and animal care complied with the principles of the laboratory animal care and with the guidelines of the International Council of Laboratory Animal Science. The total number of animals used in this study was 103 ($n_{TG} = 44$; $n_{WT} = 59$). The separation of experimental animals into specific study groups is illustrated in Table 1.

2.3. In vivo [^{18}F]FMPEP-d_2 PET imaging

Longitudinal data were obtained by in vivo PET imaging using APP/PS1-21 mice (n = 8, male) and WT control littermates (n = 8, male). The mice underwent PET scans at 6, 9, 12, and 15 months of

Table 1
The total number of mice used in the study

Genotype	Sex														
	APP/PS1-21							WT							
	M				F			M					F		
Age (mo)	6	9	12	15	6	9	12	2–4	6	9	12	15	6	9	12
In vivo PET imaging	8[a]	8[a]	8[a]	7[a]					8[b]	8[b]	8[b]	6[b]			
Ex vivo brain autoradiography	3	5	2	10	1	6	2		3	5	2	6	2	4	4
IHC	[c]	[c]	[c]	[c]	[c]	[c]	[c]		[c]	[c]	[c]	[c]	[c]	[c]	[c]
Western blot		3				4				3				3	
Radiometabolite analysis								15							
Pretreatment studies								4							

Key: F, female; M, male; PET, positron emission tomography; WT, wild-type.
[a] The same APP/PS1-21 mice.
[b] The same WT mice.
[c] The number of animals in immunohistochemistry (IHC) corresponds to the number in the ex vivo studies.