Distribution patterns of CB1 mRNA and protein in a triple transgenic mouse model of Alzheimer's disease: a longitudinal study

A. M. Lavecchia[1], G. Bedse[1], A. Romano[1], M. Elphick[2], S. Gaetani[1], T. Cassano[3], V. Cuomo[1]

[1]Dept. of Physiology and Pharmacology 'V. Erspamer', Sapienza Univ. of Rome
[2]School of Biological & Chemical Sciences, Queen Mary University of London, UK
[3]Dept. of Clinical and Experimental Medicine, University of Foggia

The endocannabinoid system (ECS) has gained much attention as a new potential pharmacotherapeutic target in various neurodegenerative diseases including Alzheimer's disease (AD). The type 1 of cannabinoid receptors (CB1) is the most abundant G protein–coupled receptor expressed in the central nervous system (CNS). Through the activation of CB1 receptors, in the CNS, the ECS exerts important functions such as retrograde inhibition of neurotransmitter release, control of neuronal excitability, and regulation of various forms of synaptic plasticity. Aberrant pattern of CB1 receptor expression and altered receptor densities have been observed postmortem in the brain of patients suffering from AD. However, these observations are still sparse and often contradictory, so that the association between CB1 alterations and the development of AD neuropathology is still unclear.

The aim of the present study was to evaluate CB1 expression in the brain of a murine model of AD at different months of age, to test whether the temporal and regional pattern of such possible alterations overlap with those of Aβ and tau pathology in this model. In particular, we used the triple transgenic mouse model of AD (3×Tg-AD), developed by Oddo *(Oddo et al., 2003 Neuron 39:409-421)*, harbouring three mutant human genes $PS1_{M146V}$, APP_{Swe}, and Tau_{p301L} and developing Aβ and tau pathology in a regional-related and age-dependent manner, with Aβ deposits manifesting at 6 month of age, prior to tangle formation.

In this study CB1 mRNA and CB1 protein levels were evaluated in 3×Tg-AD mice compared to their wild-type littermates at 2-3, 6-7 and 12 months of age, by *in-situ hybridization* and immunohistochemistry, respectively. The semiquantitative analyses of the respective signals obtained were performed in prefrontal cortex (PFC), prelimbic cortex (PrL), dorsal hippocampus (DH), basolateral amygdala (BLA) and ventral hippocampus (VH), all areas strongly affected by the neuropathology and with high CB1 densities.

At 2 months of age, we found that there was no change in CB1 mRNA and protein levels in 3×Tg-AD mice compared to Non-Tg mice in all analyzed brain areas. However at 6-7 and 12 months of age, CB1 mRNA levels were significantly higher in PFC, DH, BLA and lower in VH in 3×Tg-AD mice compared to their wild type littermates. Results obtained from CB1 immunohistochemistry revealed that CB1 protein expression was unchanged in 3×Tg-AD mice compared to Non-Tg mice at 2-3 and 6-7 months of age, while a significant decrease of CB1 receptor immunoreactivity was detected in BLA and in DH of 12-month-old 3×Tg-AD mice, with no sign of alteration in the other brain areas. Interestingly, the hippocampal decrease was evident in the CA3 subfield of DH, while the CA1/CA2 subfields resulted unaffected.

It is known that 6 months old 3×Tg-AD mice develop extracellular diffused $A\beta_{42}$ plaques in neocortex and intraneuronal $A\beta_{42}$ buildup in hippocampus, cortex and amygdala. Alterations of CB1 mRNA but not protein appear at this age and become clearly evident (involving also the protein levels) at 12 months of age, when extracellular $A\beta_{42}$ deposits are found in frontal cortex, amygdala, DH and in VH whereas tau immunoreactivity is evident only in CA1 neurons of hippocampus. At this age CB1 mRNA was increased while CB1 protein levels were decreased in BLA and in DH. We hypothesize that this discrepancy might be due to modification at translational and/or post-translational levels, although further studies are needed to address such hypothesis.

Overall our results show that 3×Tg-AD do not have inborn altered CB1 mRNA and protein expression, as they did not show any alteration at 2-3 months of age when their phenotype is still normal. The altered CB1 mRNA/protein levels appear, rather, to be age-and/or pathology-dependent, thus supporting the idea of a critical role of the ECS in AD and its possible impact as novel pharmacological target.