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Chapter 3

CONTROL OF INTRACELLULAR LEVELS OF FREE ARACHIDONIC ACID: A TARGET IN THERAPEUTIC OR PREVENTIVE STRATEGIES AGAINST ALZHEIMER'S DISEASE?

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ABSTRACT

Arachidonic acid (ARA) is one of the two most abundant polyunsaturated fatty acids (PUFAs) in brain, especially in neuronal cells, and can be a target in the fight against the major public health concern represented by Alzheimer's disease (AD) and related disorders in high- as well as in low-income countries where elders constitute a growing part of the population. The numerous studies devoted to the AD pathophysiological mechanisms emphasized the role of the amyloid-β (Aβ) oligomers generated from the precursor protein APP. Aβ oligomer-induced disruption of hippocampal synapses is recognized as the first cellular alteration in AD, leading to reduced cognitive abilities. Furthermore, the occurrence of neuroinflammation and oxidative stress is strongly linked to the AD process. But the mechanisms responsible for

AD initiation are still largely unknown, although their identification is critical to design efficient therapeutic or preventive strategies. ARA was first considered in AD as a mediator of neuroinflammation and oxidative stress through its conversion into pro-inflammatory eicosanoids by several enzymes such as 5-lipoxygenase and cyclooxygenase-2. Upregulation of these enzymes in AD brain could contribute to the pathophysiological process by amplifying neuroinflammation and by increasing Aβ oligomer production and neurotoxicity. Other studies showed that free ARA is involved in essential synaptic functions through its effects on several protein kinases or its interactions with SNARE components in the traffic of synaptic vesicles. Therefore, free intracellular ARA levels must be adjusted to physiological needs at adequate cellular locations. ARA is specifically released from membrane phospholipids by cytosolic phospholipase A_2 ($cPLA_2$-α). We and other groups demonstrated that $cPLA_2$-α is an upstream effector of the Aβ oligomer-induced apoptotic cascade, while neuroprotective effects are observed in $cPLA_2$-α KO models or in response to pharmacological inhibitors. Increase in intraneuronal free ARA levels could also result from the catabolism of endocannabinoids by monoacylglycerol lipase (MAGL). The modification of the activities of MAGL and other endocannabinoid-hydrolyzing enzymes in AD could reduce the putative positive effects of the endocannabinoid signaling. Interestingly, several acyl-CoA synthetases or lysophospholipid acyltransferases were recently characterized and could antagonize the effects of $cPLA_2$-α and MAGL on the free ARA levels in brain cells. Furthermore, ARA is believed to compete with incorporation of protective docosahexaenoic acid (DHA) into the membrane phospholipids. The unbalance between omega-3 and omega-6 PUFAs in Western diets potentially favors the increase in brain ARA pool compared to that of DHA. All these findings suggest that controlling the levels of intracellular free ARA could be of valuable interest in therapeutic or preventive strategies against AD, as we discussed in this chapter.

INTRODUCTION

Alzheimer's disease (AD) is characterized by a progressive loss of memory associated with various behavior perturbation and is the main form of senile dementia (about 60% of the cases). Apart some rare familial forms (less than 1% of the cases), the large majority of the AD forms are sporadic. The causes of these sporadic forms are still unknown and there is no clear risk factor except aging. Therefore, AD and other senile dementia constitute a major health concern not only in developed countries but also in all the low-

and mid-income level countries in which elders represent a growing part of their population. In 2010, 35.6 millions of people were affected by senile dementia and about 50% of these patients live in high-income countries. The number of affected people will exceed 65 million in 2030, with about 60% in low- and mid-income levels (International World Alzheimer report 2010). The total estimated worldwide costs of dementia are US$604 billion in 2010, i.e., around 1% of the world's gross domestic product. In front of these dramatic epidemiological predictions, there is no efficient therapeutic treatment or established preventive strategy. The fight against AD requires a better understanding of its mechanisms which are still largely unknown. Studies about AD mechanisms were historically based on the initial observation of two pathognomonic histological signs in AD patient brain and the identification of their main constituents: neurofibrillar tangles formed by the aggregation of hyperphosphorylated Tau protein and amyloid plaques resulting from the deposit of amyloid-β (Aβ) peptide. These two signs result from complex mechanisms including deregulation of protein kinases, alteration of protein maturation and transportation, synapse dysfunctions and neuroinflammation. The roles of lipids and peculiarly arachidonic acid (ARA) and its derivatives were originally deduced from the finding that neuroinflammation plays a major role in AD process. But ARA and some of its derivatives such as endocannabinoids interfere with synapse functions which are altered in the early steps of AD. Furthermore, AD also seems to modify ARA homeostasis in membrane, which can lead to enhancement of Aβ peptide production and neurotoxicity. In this chapter, we will first examine the main knowledge about the AD mechanisms by focusing on synaptotoxicity and neuroinflammation, their relationship with ARA derivatives and the various enzymes generating them. We will then study how ARA itself is involved in synaptic functions and whether the balance between its release and its incorporation into membranes could be a target in therapeutic or preventive strategies against AD.

ALZHEIMER'S DISEASE MECHANISMS: A LIPID AND MEMBRANE STORY

The Aβ peptide forming the amyloid plaques in hippocampus and cortex of AD patients is generated by the sequential cleavage of the amyloid precursor protein (APP) by two enzymes, the β- and γ-secretases (Figure 1). APP is a transmembrane protein located in synaptic membrane of neuronal

cells. Its gene is ubiquitously expressed and alternative splicing generates several forms in peripheral cells, although only a specific 695-aminoacid form is expressed in neurons. Its neuronal functions are still unclear, but there are some evidences that APP and its paralogs APLP1/2 can be involved in neuronal survival and synaptic communication (Dawson *et al.*, 1999), thereby playing an important role in synaptic plasticity and supporting memory formation and consolidation (Korte *et al.*, 2012). Alternatively, APP is hydrolyzed by α- and γ-secretases which generates the shorter and non-neurotoxic Aα-peptide (Figure 1). In physiological conditions, the hydrolysis of APP by α/γ-secretases activities would represent the main pathway compared to the amyloidogenic pathway involving β/γ-secretases (Thinakaran & Koo, 2008). Two β-secretases, BACE-1 and BACE-2 have been cloned (Dingwall, 2001), while γ-secretase activity is supported by a 3-protein complex, presenilin-1 and -2 [PS1/2], nicastrin, PEN or APH-1 (Kimberly et *al.*, 2003). Such a complex might be required to cleave APP in the hydrophobic core of the membrane, which is quite exceptional in protease activities. In addition, γ-secretase can cleave APP at two sites, subsequently releasing two distinct peptides long of 40 or 42 amino acids [$A\beta_{1\text{-}40}$ or $A\beta_{1\text{-}42}$], the first one being the major but less neurotoxic form (Thinakaran & Koo, 2008). Other minor truncated or modified Aβ peptide species are also produced in brain, but their roles in brain physiology and AD are still poorly identified. γ-secretase have other substrates than APP such as Notch (Vetrivel *et al.*, 2006). Besides β- and γ-secretases, 3 members of the ADAM metalloproteinase family have been characterized as having α-secretase activities (Vingtdeux & Marambaud, 2012). These various secretases also generate N- and C-terminal products. The N-terminal soluble product sAPPα displays neurotrophic activities (Furakawa *et al.*, 1994), while the sAPPβ peptide generated can participate to neuronal death as ligand of death receptors (Nikolaev *et al.*, 2009). This difference of biological activities is striking since these two peptides differ only by a 16 amino-acid sequence present at the C-terminal end of the sAPPα peptide. In addition, the intracellular C-terminal fragment of APP released by the γ-secretase complex can be translocated into the nucleus to act as a transcription factor (Chang & Suh, 2010). The rare AD familial forms are linked to APP gene duplication or to mutations in APP or PS genes, which induces large overproduction of Aβ peptide prone to favor Aβ oligomerization/aggregation into toxic assemblies. But whether these mutations induce gain or loss of functions is still a debate. At least some APP gene mutations modify its subcellular location, promoting its catabolism by β- and γ-secretases. Similarly, in sporadic AD, alterations of APP location and

trafficking might modify the Aβ peptide production. The non-amyloidogenic pathway of APP hydrolysis would occur at the synaptic membrane, whereas APP would interact with β- and γ-secretases in some endosome species during its recycling between the synaptic membrane and endoplasmic reticulum, thus generating Aβ peptide (Tang 2009). The importance of this recycling process in the production of Aβ peptide from APP has been emphasized by the identification of the role of SORL1 and SORLA proteins, two members of the lipoprotein receptor superfamily, in the endosomal trafficking and by the correlation of their mutations to AD occurrence risk in some populations (Rogaeva *et al.*, 2007; Willnow *et al.*, 2010; Reitz *et al.*, 2011).

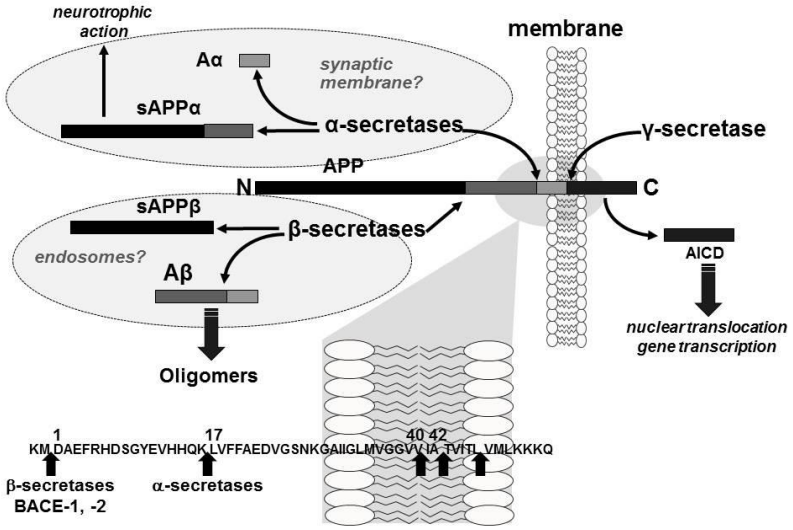

Figure 1. Hydrolysis of APP by the α-, β- and γ- secretases and the resulting peptides. Non-amyloidogenic and amyloidogenic pathways.

For a long time, most of the works about AD were focused on Aβ peptide production and its aggregation into amyloid plaques. Therefore, most of the therapeutic strategies were developed with the aim to inhibit plaque formation or to favor their disaggregation. Microglial cells and astrocytes are attracted and proliferate around the mature amyloid plaques. This neuroinflammatory reaction involves the synthesis of lipid mediators and the production of reactive oxygen species (ROS) which are assumed to induce the death of surrounding neurons. But several experimental data disfavor an exclusive role for the amyloid plaques, at least in the early steps of the disease. For example,

some murine AD models expressing the mutated human APP gene display cognitive and synaptic cellular alterations a long time before the occurrence of the first amyloid plaques (Hsia *et al.*, 1999; Mucke *et al.*, 2000; Wu *et al.*, 2004). In humans, the number of plaques and their content in Aβ peptide do not correlate with the severity of the symptoms, which on the contrary depends on the brain concentrations of soluble Aβ oligomers (Mc Lean *et al.*, 1999). It is now well admitted that these oligomers are the main agents of AD, especially in early AD (Drouet *et al.*, 2000; Walsh & Selkoe, 2004). Aβ peptide forms various oligomer species, from dimers, trimers to higher molecular range species such as dodecamers (Larson & Lesné, 2012) or Aβ-derived diffusible ligands (ADDLs) (Krafft & Klein, 2010). Several oligomeric species are formed in human brain during aging, target synaptic membranes in which they colocalize with the PSD95 complex (Lacor *et al.*, 2004) and induce the first synaptic dysfunctions (Rowan *et al.*, 2007). Some authors postulate that dimers are the main agents of AD (Jin *et al.*, 2011), while others assume that several species contribute to the whole neurotoxic effect in AD (Klyubin *et al.*, 2012). Although it is clear that interactions of Aβ oligomers with neuronal membranes are critical for their cellular effects, two hypotheses are still being debated for the mediation of the neurotoxic effects of Aβ oligomers: either the existence of putative membrane receptors, either a direct modification of the membrane organization itself. Regarding the latter hypothesis, Aβ peptide oligomers can induce the fusion of membranes (Pillot *et al.*, 1996) and this fusogenic ability is based on the peculiar conformation of the peptide in solution (Morgan *et al.*, 2004). In addition, Aβ peptide oligomers are able to form channels in the membranes of several cell types including neurons (Fraser *et al.*, 1997). These channels allow calcium influx which initiates several pro-apoptotic pathways (Kagan *et al.*, 2002). Several putative receptors have also been described for the Aβ oligomers, including the prion protein [PrPc] (Laurén *et al.*, 2009) and the APP protein itself or its paralogs (Shaked *et al.*, 2006). The fact that Aβ oligomers represent very complex mixtures in solution does not facilitate the confirmation that these putative receptors effectively mediate the Aβ peptide effects. Finally, it is worthy to remind that Aβ peptide is generated within the neuronal cells and an endoplasmic reticulum stress or mitochondrial dysfunctions are induced by these intraneuronal Aβ oligomers (Umeda *et al.*, 2011). After their production, Aβ peptide is degraded in the neuronal lysosomes or extracellularly by neprilysine or insuline degrading enzyme [IDE] (Wang *et al.*, 2006). Aβ peptides are also exported to blood through the blood-brain barrier (BBB) to avoid its brain accumulation. Several works showed the involvement of

lipoprotein receptor family members such as LRP1 in this exportation processes (Sagare *et al.*, 2007).

Neurofibrillary tangles are correlated with the severity of AD and their number and extent in the brain are the basis of the Braak classification of AD evolution (Serrano-Pozo *et al.*, 2011). They are formed inside the neuronal cells by aggregation of the various Tau isoforms. These Tau isoforms are members of the microtubule-associated proteins (MAP) which constitute the neuronal cytoskeleton. Hyperphosphorylation of Tau on several non-physiological sites modifies its conformation and causes its aggregation in neurofibrillary tangles (Köpke *et al.*, 1993). The resulting disorganization of the axonal transportations from the nucleus to the synapse leads to the neuronal degeneration and death. Tauopathies are present in various neurodegenerative diseases and senile dementia (Brandt *et al.*, 2005). The signaling pathways leading to Tau hyperphosphorylation in AD are still elusive and should be the result of several dysregulations. Aβ oligomers affect Tau phosphorylation through several pathways. For example, the PrPc protein binds to Aβ oligomers and Fyn at the dendritic spines and the formation of this complex activates the Tau phosphorylation by Fyn (Wang *et al.*, 2013a). The partitioning defective-1 (PAR-1) and the microtubule affinity-regulating kinases (MARK) have also been identified as mediating the effect of Aβ oligomers on Tau phosphorylation and toxicity on synapses (Yu *et al.*, 2012). The mice expressing the E963Δ APP mutant accumulate low molecular-weight Aβ oligomers and display hyperphosphorylated Tau isoforms inside the neuronal cells at the age of 8 months (Tomiyama *et al.*, 2010).

As shown above, Aβ oligomers are critical agents of AD whose production and neurotoxic effects mostly rely on membrane constituents and functions. Furthermore, it is important to notice that AD progresses over several years or decades. One or several yet unidentified events lead to Aβ accumulation in the brain through its overproduction or decrease or its clearance. This results in the early synaptic dysfunctions and then in the initiation of a vicious cycle involving Aβ oligomer accumulation, neuroinflammation, vascular alterations and neuronal cell death (Figure 2). Metabolic diseases such as obesity and diabetes increase the risk of occurrence of AD (Carlsson, 2010; Luchsinger *et al.*, 2012). These diseases involve dysregulation of several hormones, insulin, ghrelin and leptin, which also contribute to the regulation of Aβ production. Metabolic diseases also involve low-levels of general inflammation with circulating inflammation markers (Choi *et al.*, 2013). It has been shown that the induction of peripheral inflammation can stimulate neuroinflammation in brain which could contribute

to the initiation of neurodegenerative diseases such as AD. Prostaglandins and leukotrienes are produced by ARA conversion and are powerful mediators of inflammation. Furthermore, the endocannabinoids, which are also ARA derivatives, are synaptic retro-messengers. How these various molecules participate to the AD process is described below.

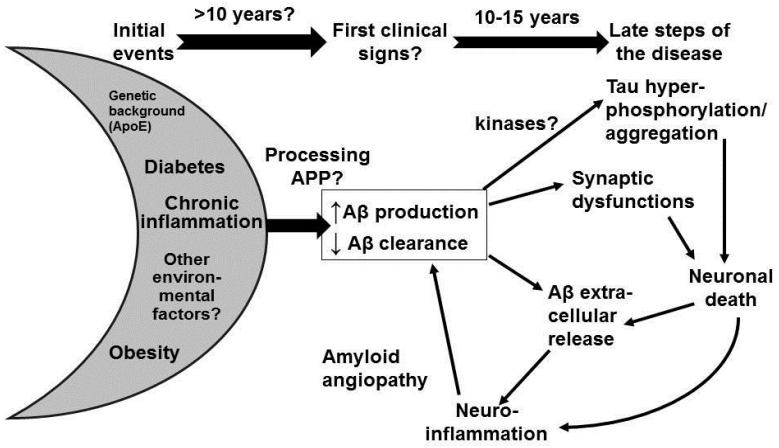

Figure 2. Risk factors and interplay of the various pathological events in AD.

ROLE OF ARACHIDONIC ACID DERIVATIVES IN ALZHEIMER'S DISEASE: LEUKOTRIENES, PROSTAGLANDINS AND ENDOCANNABINOIDS

ARA is converted by the cyclooxygenase-1 (COX-1) and -2 (COX-2) enzymes into prostaglandin PGH2 which is the substrate of various prostaglandin synthases to give the diverse prostaglandins. The involvement of this pathway was first suggested by the fact that long-term treatment by nonsteroidal anti-inflammatory drugs (NSAIDs) decreases the AD incidence (In't Veld *et al.*, 1998). Since NSAIDs are potent inhibitors of COX-1 and -2, many studies were devoted to the expression and activity of these enzymes. COX-2 is usually seen as an inducible enzyme whose expression is increased in inflammation in most of the tissues and organs. However, brain is exceptional in the sense that COX-2 mRNA and protein are constitutively

expressed at least in some neurons and during development (Malinska *et al.*, 1999; Minghetti, 2004), which indicates that this enzyme is required for basic functions such as synaptogenesis and memory consolidation. Lukiw & Bazan (1997) first observed a trend for higher COX-2 mRNA levels in the neocortex of 10 AD patients compared to 15 control subjects, but noticed in both groups large individual variations that they associated with differences in inflammatory processes. Thereafter, Yasojima et al., (1999) described an overexpression in pyramidal neurons of AD patients. Ho et al., (2001) and Fujimi et al., (2007) studied immunoreactivity of COX-2 in hippocampus of several tens of AD patients and control subjects and confirmed a COX-2 overexpression in pyramidal neurons of AD patients in this brain region. Both research groups found a correlation between the severity of the disease and the level of COX-2 overexpression in CA1 to CA3 subdivisions of hippocampus, but differed on the precise subdivision in which this correlation was established, CA1 (Fujimi et al., 2007) or CA2/CA3 (Ho et al., 2001). COX-2 overexpression in neuronal cells suggests that this enzyme is involved in neuronal AD-dependent mechanisms rather than in glial cell-dependent inflammatory mechanisms as initially assumed. Indeed, $A\beta_{1\text{-}42}$ oligomers do not stimulate the COX-2 expression in human microglial cell primary cultures (Hoozemans *et al.*, 2002) and microglial cells around amyloid plaques display COX-1 immunostaining (Yermakova et al., 1999). Eriksen et al., (2003) showed that non selective COX-1/2 inhibitors reduce $A\beta_{1\text{-}42}$ peptide in mice expressing human APP gene by targeting γ-secretase activity. Overexpression of COX-3, a splice variant of COX-1 and putative target of acetaminophen (Chandrasekharan *et al.*, 2002) has also been reported in AD hippocampus and $A\beta_{1\text{-}42}$ treated neuronal primary culture neuronal cells (Cui et al., 2004). Boutaud et al., (2002) demonstrated that PGH2 prostaglandin favors the *in vitro* oligomerization of $A\beta_{1\text{-}42}$ peptide and form levuglandin-adducts with the peptide, which indicates that prostaglandins might influence AD process in several ways.

PGH2 is mainly converted into PGE2 and PGD2 in brain by various prostaglandin E and D synthases. The main β-secretase BACE-1 also cleaves the membrane-bound prostaglandin E2 synthase-2 (mPGES-2) in its N-terminal end, which results in enhancement of PGE2 production (Kihara *et al.*, 2010). PGE2 produced in neuronal cells in such a way can amplify neuroinflammation since PGE2 was shown to induce IL-6 production in astrocytes (Fiebich *et al.*, 1998). By contrast, the microsomal PGE2 synthase-1 directly regulates microglial proliferation around amyloid plaques since its gene deletion in the TG2576 murine AD model decreases this proliferation

and attenuates the cognitive impairments of the transgenic mice (Akitake *et al.*, 2013). PGD2 is overproduced in neuronal cells of AD patients (Iwamoto *et al.*, 1989) as well as in glial cells surrounding the amyloid plaques which overexpress the hematopoietic type of PGD2 synthase and the PGD2 receptor DP1 (Mohri *et al.*, 2007). The various production sites and targets of PGE2 and PGD2 illustrate the fact that several types of cooperation between neuronal and glial cells contribute to the extension of AD. The binding of the other lipocalin-type PGD2 synthase to Aβ peptide in CSF must be reminded. This enzyme was considered as a chaperone for Aβ peptide (Kanekiyo *et al.*, 2007; Lovell *et al.*, 2008; Hansson *et al.*, 2009), although it has probably no relationship with ARA conversion into PGD2.

Leukotrienes are the second group of eicosanoids. Their production is initiated by the conversion of free ARA by 5-lipoxygenase (5-LOX) into 5-hydroxyperoxyeicosatetraenoic acid [5-HPETE], similarly to the synthesis of PGH2 by COX-1/2. 5-HPETE will be then stabilized into 5-hydroxyeicosatetraenoic acid (5-HETE) or converted by 5-LOX into leukotriene A4 (LTA4), which is used as a substrate either by LTA4 hydrolase to give leukotriene B4 (LTB4), or by glutathione *S*-transferase to produce leukotriene C4 (LTC4). LTC4 can be further converted into leukotrienes D4 and E4 (LTD4, LTE4). In addition, the presence of an accessory 5-LOX activating protein (FLAP) is required for the optimal conversion of ARA by 5-LOX without direct interaction between the two proteins (Vickers, 1995). Ikonomovic et al., (2008) studied a small series of 11 AD and 5 control patients and described an overexpression of 5-LOX in neuronal and glial cells of the temporal cortex. But most evidences of the involvement of 5-LOX in AD were provided by studies using either 5-LOX pharmaceutical inhibitors or 5-LOX knockout mice. As soon as 1994, Goodman et al., (1994) showed that two 5-LOX inhibitors, nordihydroguaiaretic acid and AA861, protect rat hippocampal neuronal primary cultures against Aβ neurotoxicity and decrease free Ca^{2+} influx and ROS production. The administration of zileuton, another 5-LOX inhibitor, to transgenic AD model showed that 1) 5-LOX increases Aβ production by activating the CREB transcription factor and by enhancing the transcription of the constituents of the γ-secretase complex through activation (Chu & Pratico, 2011a, b), 2) 5-LOX also stimulates cdk-5 activity and expression levels, which induces Tau hyperphosphorylation (Chu & Pratico, 2013; Chu *et al.*, 2013b, c). In these studies, the administration of zileuton attenuated both amyloid and Tau pathologies observed in these murine AD models and improved their cognitive deficits. Pratico's group also demonstrated that the specific FLAP inhibitor MK-591 reduces Aβ

overproduction and Tau hyperphosphorylation in the TG2576 mouse (Chu & Pratico, 2012; Chu *et al.*, 2013a), although this AD model is mainly based on the expression of the human Swedish mutant APP gene. This group confirmed the involvement of 5-LOX and FLAP in Aβ production and cdk5-mediated Tau phosphorylation by overexpressing 5-LOX gene (Chu *et al.*, 2012a, 2012b, 2013c) or by deleting the 5-LOX and FLAP genes in transgenic AD model mice (Firuzi *et al.*, 2008; Puccio *et al.*, 2011; Giannopoulos *et al.*, 2013a,b; Joshi et al., 2013a). However, by treating 3X-transgenic mice by LPS, they evidenced that 5-LOX gene deletion attenuates the worsening effect of LPS-induce neuroinflammation on Aβ production but not that on Tau hyperphosphorylation and aggregation (Joshi *et al.*, 2013b). Although the previous data were reported by the same groups, some other groups used other pharmaceutical inhibitors and concluded to the involvement of 5-LOX and FLAP in AD process. Valera et al., (2013) used a pyrazole called CNB-001 which lowers Aβ production in AD transgenic mice and suggested that 5-LOX mediates endoplasmic reticulum dysfunction. Hawkes et al., (2014) treated mice expressing a double mutant APP gene with the MK886 FLAP inhibitor and observed reduced expression levels of nicastrin, a member of the γ-secretase model. 5-LOX overexpression and LTB4 overproduction were also found in peripheral blood mononuclear cells of AD patients and were associated with reduced methylation of 5-LOX promoter DNA (Di Francesco, 2013). Furthermore, protective effects of 12-lipoxygenase inhibition have also been reported, but only on cortical neuronal cell primary cultures (Lebeau *et al.*, 2001, 2004). Pratico's group evidenced that LTB4 increases Aβ production and γ-secretase expression level in neuronal cell primary cultures (Joshi *et al.*, 2014). But Wang et al., (2013b) demonstrated that LTD4 also stimulates Aβ production in neuronal cell cultures through enhancement of β- and γ-secretase activities and these effects depends on the activation of the NF-κB signaling pathway. Therefore, since 5-LOX catalyzes the upstream reaction in the leukotriene cascade synthesis, other works are required to identify the nature of the ARA derivative(s) (leukotriene(s) or HETE(s)) which is(are) the *in vivo* causative agent(s) of these various effects.

Endocannabinoids are not direct derivatives of ARA, on the contrary to prostaglandins or leukotrienes, but ARA is one component of these complex molecules. Endocannabinoids include two signaling lipids, N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG). AEA is synthetized in two steps, 1) the transfer of ARA from an 1-arachidonic acid phosphatidylcholine to the polar end of a phosphatidylethanolamine with the formation of an amino-ester bound, 2) the cleavage of this lipid by a specific

phospholipase D which releases AEA. 2-AG is also produced in two steps 1) the hydrolysis of a 2-arachidonyl-PI by a PI-specific phospholipase C, 2) the hydrolysis of the resulting diacylglycerol by a diacylglycerol lipase (Wang & Ueda, 2009). Only a few studies were devoted to endocannabinoid concentration in AD. No variation of circulating endocannabinoid concentration was found (Koppel *et al.*, 2009), but significantly lower levels of the endocannabinoid anandamide and its precursor 1-stearoyl, 2-docosahexaenoyl-sn-glycero-phosphoethanolamine-N-arachidonoyl (NArPE) were recently measured in frontal and temporal cortex of 38 AD patients compared to 17 control subjects without any difference for 2-AG (Jung *et al.*, 2012). By contrast, protective effects of endocannabinoids against Aβ neurotoxicity were reported in numerous studies. The first studies used neuronal cell primary cultures or on neuroblastoma cell lines to show *in vitro* neuroprotection by cannabidiol, and the two endocannabinoids (Milton, 2002; Esposito *et al.*, 2006a, b; Noonan *et al.*, 2010; Chen *et al.*, 2011, Janefjord *et al.*, 2013). Several of these studies involved the CB1 receptor, one of both G-protein coupled receptors of endocannabinoids, in this neuroprotective effects (Milton, 2002; Noonan *et al.*, 2010; Chen *et al.*, 2011). But *in vivo* studies yielded equivocal results about the role of CB1 receptor in the protection against Aβ peptide. Pamplona et al., (2012) showed that lipoxin A4, a product of ARA conversion by 15- and 5-lipoxygenase, enhanced the binding of anandamide to CB1 and decreased the Aβ-induced cognitive alterations in mice. ACEA, a specific CB1 agonist, decreased inflammation around amyloid plaques in a double mutant APP/PS1 mouse line (Aso *et al.*, 2012) and preserved the electrophysiological functions of rat hippocampus pyramidal neurons after cortical Aβ injections (Aghani *et al.*, 2012). However, Stumm et al., (2013) showed by breeding mutant APP–bearing with CB1 deficient mice that reduction of CB1 expression reduces amyloid load and inflammation, but worsens cognitive deficit. In addition, measurement of CB1 expression led to contradictory results in AD patient brains or in AD model mice (Westlake *et al.*, 1994; Lee *et al.*, 2010; Kalifa *et al.*, 2011; Mulder *et al.*, 2011; Kärkkäine *et al.*, 2012; Ahmad *et al.*, 2013). On the contrary to CB1, CB2 receptor was found to be overexpressed in AD microglial cell in several studies (Benito *et al.*, 2003; Nunez *et al.*, 2008) and its stimulation by specific agonists have positive effects on amyloid load, neuroinflammation, tauopathy and cognitive abilities in AD model mice (Martin-Moreno, 2012; Solas *et al.*, 2013; Wu *et al.*, 2013; Aso *et al.*, 2013). Another endocannabinoid-associated neuroprotective mechanism was indicated by Bachmeier et al., (2013) who found that endocannabinoids increase LRP-1 expression level in BBB and

subsequently the clearance of Aβ peptide. Only a few studies compared the respective effects of 2-AG and AEA, the first one being quantitatively the major endocannabinoid in brain. AEA, but not 2-AG, increases the expression of nicastrin and favors the Notch signaling pathway, thereby promoting the $A\beta_{1-40}$ over the $A\beta_{1-42}$ production in neuronal cell primary cultures (Tanver *et al.*, 2012). On the contrary, only 2-AG protects the human neuroblastoma SH-SY5Y cell line against the Aβ-induced apoptosis (Janefjord, 2013). Whether the discrepancy between these two studies might be due to the different cell models remains to be determined.

The F2 isoprostanes are the last group of ARA derivatives which can be measured in AD patients. These compounds result from the oxidation of ARA, which also affects other PUFAs, mainly DHA which leads to neuroprostanes [or F4-isoprostane]. Higher levels of both types of compounds were measured in AD brain as well as in other neurodegenerative diseases (Montine *et al.*, 2004). Moreover, ARA and DHA undergo oxidation in membrane phospholipids (Morrow *et al,*. 1992) and are released by type-II secretory phospholipases A_2 and PAF acetyl hydrolases (Tselepis & Chapman, 2006). They correspond to the non-specific oxidative stress associated to AD as to other neurodegenerative disease and do not seem to be causative agents of AD.

We have seen that several studies reported overexpression of enzymes converting ARA into prostaglandins or leukotrienes in patients or in AD model mice, although there are some conflicting results in patients, probably due to the restricted size of the series and to the difficulty to handle *post-mortem* samples. This overexpression indicates the involvement of these enzymes and their products in AD (Figure 3). Studies on cellular models or on transgenic mice indicate that these pathways participate not only to the extension of the neuroinflammatory processes, but also to the key mechanisms of AD, i.e., Tau hyperphosphorylation and Aβ overproduction or reduction of its clearance. Taken together, these studies provided the preclinical basis for testing 5-LOX or COX inhibitors in treatment of AD patients. But conventional NSAIDs did not display any efficiency against AD in clinical trials, at least after the establishment of the disease. Therefore, clinical studies are required to evaluate the early interest of COX-2 or 5-LOX specific inhibitors. On the contrary to eicosanoids, endocannabinoids display positive effects on neuroinflammation and key AD mechanisms on cell and AD model mice. Additional studies are necessary to determine the respective contribution of the pathways depending on CB1 or CB2 receptors and a better elucidation of these pathways. AD therapy could benefit of the finding of new specific agonists devoid of psychomimetic effect. Eicosanoid synthesis is initiated by

the release of free ARA which can be considered as a key step for the production of the various prostaglandins and leukotrienes. Furthermore, endocannabinoids degradation generates free ARA. Therefore, limitation of release of free ARA could have beneficial effect not only to treat but also to prevent AD. The relationship between AD and the enzymes releasing ARA from membrane phospholipids and endocannabinoids will be examined below.

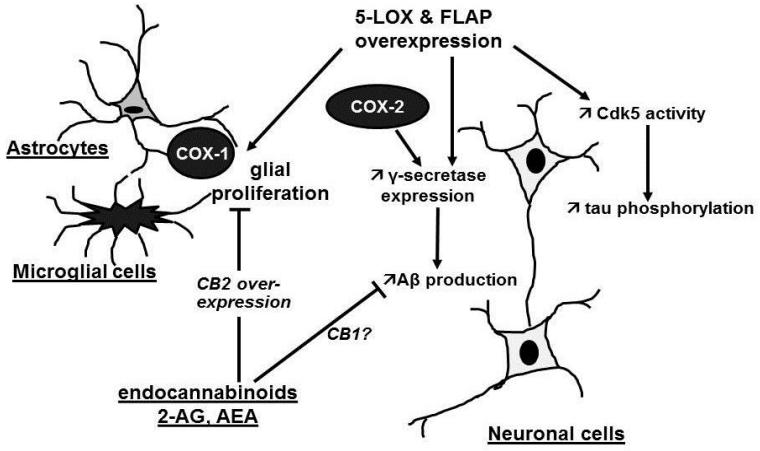

Figure 3. Roles of 5-LOX/FLAP and COX1/2 on AD mechanisms. Endocannabinoids counteract the eicosanoid effects. Overexpression and role of CB2 receptors in glial cells have been reported in several studies whereas conflicting results have been published about CB1 expression levels and role in AD.

SOURCE OF FREE ARACHIDONIC ACID IN BRAIN AND ALZHEIMER'S DISEASE

The main cellular source of free ARA is the release of this PUFA from membrane phospholipids by phospholipases A_2. These proteins constitute a superfamily which includes intracellular as well secretory and Ca^{2+}-dependent or -independent enzymes (Burke & Dennis, 2009). Before 2000, total phospholipase A_2 activities were measured and found reduced in brain cortex of AD patients (Gattaz *et al.*, 1995), but no indication about the identity of the enzymes was provided. In a more recent period, immunoreactivity for the secretory phospholipase A_2 type IIA ($sPLA_2$-IIA) was observed in astrocytes of AD patient hippocampus and inferior temporal gyrus with a stronger

intensity in the astrocytes around amyloid plaques (Moses *et al.*, 2006). These data are consistent with the role of $sPLA_2$-IIa in inflammation (Burke & Dennis, 2009) and its expression in astrocyte cultures (Oka & Arita 1991). Indeed, these authors confirmed that interleukin-1 and $A\beta_{1-42}$ peptide induce the expression of $sPLA_2$-IIA mRNA in human astrocyte cultures (Moses *et al.*, 2006). Treatments of neuronal cell cultures by exogenous ARA or another secretory phospholipase A_2, PLA_2 type III, increased the localization of APP at the membrane surface, its degradation by α-secretase and reduced the Aβ production (Yang *et al.*, 2010). These data were reminiscent to those of previous studies about the processing of APP in CHO cells expressing this protein and the m1 muscarinic receptor (Emmerling *et al.*, 1993, 1996). But these secretory PLA_2 release all the fatty acids from the sn-2 position of membrane phospholipids without any specificity for ARA. A calcium-dependent cytosolic phospholipase A_2, named $cPLA_2$-α or $cPLA_2$ type IVA according Dennis & Burke's classification, is the only phospholipase A_2 which specifically used as substrate phospholipids bearing ARA at their sn-2 position. Translocation of this cytosolic enzyme to membranes requires phosphorylation on its Ser^{505} by the MAP-kinase cascade although phosphorylation of other sites by other kinases has been observed (Hirabayashi *et al.*, 2004). Stephenson et al., (1996) suggested the involvement of $cPLA_2$-α in AD by describing a stronger immunoreactivity of this protein with a monoclonal antibody in 5 AD patient cortex compared to 6 control subjects. This immunoreactivity colocalized mainly with GFAP with a higher intensity around amyloid plaques, indicating an expression of $cPLA_2$-α in astrocytes and its association with neuroinflammation. This result was consistent with the previously described expression of $cPLA_2$-α in human astrocytes (Stephenson *et al.*, 1993). Microglial cells also contribute to $cPLA_2$-α overexpression in human and mice in several brain pathologies including AD (Stephenson *et al.*, 1999). The increased expression of $cPLA_2$-α in AD hippocampus was confirmed by Lukiw (2004) in mRNA microarrays experiments and was related to the overexpression of other neuroinflammatory markers including COX-2. The association between $cPLA_2$-α and AD process in glial cells was thereafter supported by several studies on astrocytes and microglial cell cultures (Zhu *et al.*, 2006; Hicks *et al.*, 2008; Szaingurten-Solodkin *et al.*, 2009). These works established that $A\beta_{1-42}$ peptide-induced activation of NADPH oxidase in these cells leads to ROS production and $cPLA_2$-α phosphorylation and translocation to membranes, peculiarly to mitochondria membranes which play a major role in oxidative stress (Zhu et al., 2006). In addition, $A\beta_{1-42}$ peptide also increases expression of $cPLA_2$-α and COX-2

through the activation of the redox-sensitive NF-κB transcription factor, at least in microglial cells (Szaingurten-Solodkin, 2009). Reciprocally, inhibition of the $cPLA_2$-α expression decreases NADPH oxidase activation, NO production and modifications of astrocyte membrane (Szaingurten-Solodkin, 2009; Hicks *et al.*, 2009). The strongly linked activations of $cPLA_2$-α and NADPH oxidase by $A\beta_{1-42}$ peptide suggest a common upstream step in the initiation of the $A\beta_{1-42}$ signaling pathway. Askarova et al., (2011) reported that the receptor for advanced glycation endproducts [RAGE] binds $A\beta_{1-42}$ peptide on the plasma membranes of astrocytes and endothelial cells and triggers the downstream events. RAGE has been previously described as a major actor in the mediation of $A\beta_{1-42}$ peptide effects and its homeostasis in brain (for review, see Deane *et al.*, 2004). Activation of $cPLA_2$-α and MAP-kinase cascade are triggered in retina pericytes like in endothelial cells, which underlines the vascular effects of Aβ oligomers (Anfuso *et al.*, 2004, Nicotra *et al.*, 2005).

Although it has not been initially noted, $cPLA_2$-α is also expressed in neuronal cells in which it mediates the neurotoxic effects of $A\beta_{1-42}$ peptide. In 2005, Kriem et al., (2005) demonstrated for the first time that the reduction of the $cPLA_2$-α activity or expression can protect the neuronal cells in primary cultures from the Aβ peptide-induced apoptosis. The $cPLA_2$-α activation pathways induced by Aβ peptide were investigated and the critical role of the MAP-kinase cascade was evidenced by using Erk1/2 and p38 inhibitors. These results were obtained by using either $A\beta_{1-40}$ or $A\beta_{1-42}$ oligomers although the last type is considered as much more toxic. Bate & William (2010) described that $A\beta_{1-42}$ peptide decreases the expression level of synaptophysin, the recycling of synaptic vesicles in mouse cortical neuronal cell cultures and phosphorylation of $cPLA_2$-α. This illustrates the synaptotoxicity of this peptide in a cell model and the involvement of $cPLA_2$-α which was considered by the authors as a presynaptic marker. These authors also observed that when previously mixed with $A\beta_{1-42}$, $A\beta_{1-40}$ peptide decreases $A\beta_{1-42}$ effects including $cPLA_2$-α activation. The discrepancy between the results of the two studies regarding $A\beta_{1-40}$ effect could be related to the fact that the first study described neurotoxic effects of $A\beta_{1-40}$ at 1 µM concentrations while the second one found neuroprotective at ten-fold lower concentrations. As in glial cells, NADPH synthase regulates $cPLA_2$-α activity in response to Aβ peptide in neuronal cell culture and NADPH oxidase is controlled by NMDA receptors in these neuronal cells since antagonists of both NMDA receptors and NADPH oxidase decrease the Aβ-induced release of ARA and $cPLA_2$-α phosphorylation (Shelat *et al.*, 2008). This work confirmed that MAP kinases

are activated by Aβ peptide in neuronal cells as previously reported (Kriem *et al.*, 2005). Expression of wild-type or mutant human APP in neuroblastoma PC12 cells, which produces intracellular Aβ peptide, leads to activation of neutral NO synthase, $cPLA_2$-α phosphorylation and ARA release. This ARA release was reduced by NO synthase and γ-secretase inhibitions, indicating that the neuronal $cPLA_2$-α dependent release of ARA occurs in the frame of an inflammatory process that includes the usual partners of this reaction (NO synthase, NADPH oxidase). $cPLA_2$-α is also upregulated in neuronal cells from p25 overexpressing mice (Sundaram *et al.*, 2012). This finding is noticeable since p25 activates cdk5, which was involved in the dysregulated hyperphosphorylation of Tau. This result can be linked to those of another group which used the MAFP as *in vitro* and *in vivo* $cPLA_2$-α inhibitor (Schaeffer *et al.*, 2011). However, the *in vivo* specificity and efficiency of MAFP are questionable, since MAFP also inhibits calcium–independent PLA_2 and the endocannabinoid hydrolyzing enzymes [see below]. More specific and potent inhibitors of $cPLA_2$-α should be used to confirm these data. Such $cPLA_2$-α inhibitors have been rarely used in animal assay and might be unstable at least for some of them (Fabian *et al.*, 2013).

The way by which $cPLA_2$-α-released ARA acts to contribute to AD process was investigated in several studies. COX-2 inhibition limits the pro-apoptotic effects of Aβ oligomers by 50%, but the 5-LOX putative contribution was not explored by the authors (Kriem *et al.*, 2005). High levels of free ARA, several epoxyeicosatrienoic; diepoxyeicosatrienoic acids and 12-HETE, but no other prostaglandins and leukotrienes, were measured in brain hippocampus and cortex of mice expressing mutant APP gene (Sanchez-Meija *et al.*, 2008). High levels of phosphorylated $cPLA_2$-α were found in the hippocampus of these AD model mice as well as in the hippocampus of AD patients. By contrast, lipidomics experiments found that lysophosphatidylcholine, the other product of the phosphatidylcholine hydrolysis by $cPLA_2$-α, initiate neuroinflammation and astrogliosis induced by p25 overexpression (Sundaram *et al.*, 2012). Activation of neutral and acid sphingomyelinases is another rapid downstream event of $A\beta_{1\text{-}42}$-induced $cPLA_2$-α activation in neuronal cell primary cultures, which could be inhibited by sphingosine-1 phosphate (Malaplate-Armand *et al.*, 2006). A single intracerebroventricular injection of 1 nanomole of $A\beta_{1\text{-}42}$ oligomers activates neutral and acid sphingomyelinases and drastically reduces Akt/PKB phosphorylation and expression of synaptic markers (Desbène et al., 2012). As a result, short- and long term memory abilities of the treated mice were deeply altered within the 2-week period following $A\beta_{1\text{-}42}$ injection (Desbène *et al.*,

2012). Neuronal activation of $cPLA_2$-α would increase the localization of the non-NMDA glutamate AMPA receptor at the membrane surface and this would be the way that this enzyme contributes to Aβ neurototoxicity (Sanchez-Meija *et al.*, 2008). Breeding $cPLA_2$-$\alpha^{-/-}$ and mutant APP gene expressing mice attenuate the memory deficits, reduce the eicosanoid concentration in cortex and hippocampus and increase the survival of the resulting mice compared to the AD parent mice (Sanchez-Meija *et al.*, 2008). Interestingly, APP expression is reduced in hippocampus and neuronal cells of $cPLA_2$-$\alpha^{-/-}$ mice. Taken together with the resistance of these mice to $A\beta_{1-42}$ neurototoxicity and the fact that APP expression induce sensitivity to the $A\beta_{1-42}$ oligomers in HEK293 cells (unpublished results, this result suggests that APP could be a receptor for these oligomers. Shaked et al., (2006) previously reported that $A\beta_{1-42}$ oligomers interact with APP extracellular domain.

The degradation of endocannabinoids, after their reuptake by neuronal cells, is the second source of ARA. On one hand, the fatty acid amide hydrolase (FAAH) hydrolyses all the N-acylethanolamines, including AEA which is a minor product in this family (Wang *et al.*, 2009; Benito *et al.*, 2012). On the other hand, the major endocannabinoid 2-AG is mainly degraded in brain by monoacylglycerol lipase (Schlosburg *et al.*, 2010, Pan *et al.*, 2011). Higher expression and activity of FAAH was found in AD patients in association to a decrease in DNA methylation of FAAH promoter (D'Adario *et al.*, 2012). Therefore, one could expect that reduction of FAAH expression or activity attenuates inflammation in patients or AD cell and animal models. However, Benito et al., (2012) observed that astrocytes from $FAAH^{-/-}$ mice are highly responsive to 5 μM $A\beta_{1-42}$ oligomers in terms of induced expression of IL-1β, COX-2, NO synthase, NF-κB and other inflammatory markers. AEA as well as other N-acylethanolamine partially reversed the $A\beta_{1-42}$–induced proinflammatory effects. But the authors failed to block the induction of inflammatory markers by $A\beta_{1-42}$ oligomers by using CB1 or CB2 receptors antagonist. By contrast, they found increased mRNA levels of the transient receptor potential vanilloid-1 [TRPV1] which binds N-acylethanolamines. In addition, treatments of wild-type astrocytes by pharmaceutical FAAH inhibitors did not reproduce an inflammatory phenotype. These results suggest that the inflammatory reaction observed in $FAAH^{-/-}$ astrocytes in presence of $A\beta_{1-42}$ oligomers results from an adaptive phenotype and the actions of several N-acylethanolamines and not only from the lack of AEA degradation. No other study on the consequences of modification of FAAH activity in AD cell or animal models has been published until now to our knowledge. Regarding the second

endocannabinoid-degrading enzyme, the $MAGL^{-/-}$ mice exhibit large modifications of synaptic functions, some of them being related to CB1 desensitization by high levels of 2-AG (Pan *et al.*, 2011). The $MAGL^{-/-}$ mice display better abilities in learning tasks compared to the wild type animals (Pan *et al.*, 2011). Chen et al., (2012) used 5X-FAD mice bearing 3 and 2 mutations of the human APP and PS1 genes, respectively, and administrated intraperitoneally the MAGL inhibitor JZL184 for 8 weeks in 4-month old animals. This treatment reduced by more than two third the total surface of amyloid plaques in cortex and hippocampus, drastically decreased the $A\beta_{1-42}$ production in cortex and at a lower extent in hippocampus. It also normalized the expression of BACE-1 which was increased in transgenic mice compared to the control animals. In addition, JZL184 administration preserved the synaptic structure and expression of NMDA and AMPA receptors in transgenic mouse hippocampi. However CB1 or CB2 receptors do not mediate the effect of MAGL inhibition since animals resulting from the breeding of CB1 or CB2 KO were still sensitive to JLZ184 treatment. Another group (Piro *et al.*, 2012) showed that MAGL gene suppression in mice leads to enhancement of 2-AG and drastic reduction of free ARA brain concentrations. The eicosanoids PGE2, PGD2 and TXB2 were also drastically reduced in $MAGL^{-/-}$ mouse brains. Furthermore, lipidomic analysis of PAD PS1/APP mouse brains revealed high concentrations of free ARA, PGE2, PGD2 and TXB2 as well as a reduction of 2-AG associated with a compensation by AEA. Breeding these animals with $MAGL^{-/-}$ mice suppressed the enhancement of ARA and eicosanoids concentrations induced by APP/PS1 mutations and normalized the 2-AG concentrations compared to the $MAGL^{-/-}$ mouse brains. MAGL inactivation also decreased Aβ production, amyloid plaque formation and neuroinflammation associated with APP/PS1 mutations. Finally, treatments by JZL184 or a CB1/CB2 receptors antagonist for 16 days suppressed production of pro-inflammatory cytokines and eicosanoids in brain of 6-month old APP/PS1 mice. Taken together, these results showed that increased degradation of 2-AG orientates free ARA to the synthesis of pro-inflammatory eicosanoids, which amplifies neuroinflammation in AD mice. Lastly, evidence about a role for MAGL in AD patients were provided by a third group (Piro *et al.*, 2012) who showed that MAGL expression correlates with Braak stages in AD patients and AD induces MAGL relocalization from cortical membranes to cytosol. These authors also reported that the expression of another 2-AG degrading enzyme, the ABHD6 serine hydrolase, was increased in microglia around amyloid plaques and decreased in hippocampal pyramidal neurons of AD patients.

All these results established that $cPLA_2$-α and MAGL are the main enzymes providing free ARA in glial and neuronal cells and play a putatively major role in AD. These enzymes are overexpressed and/or activated in hippocampal and cortical regions of AD patient brains. Experiments with pharmaceutical inhibitors in wild-type and transgenic mice also supported the involvement of these enzymes. However, additional studies are required to elucidate the ways that $cPLA_2$-α and MAGL cooperate with the downstream eicosanoids enzymes in the synthesis of the proinflammatory prostaglandins and leukotrienes. Furthermore, whether and how ARA could play a role without being converted into eicosanoid, remains to be investigated. The contribution of lysophosphatidylcholine or other lysophospholipids resulting from the hydrolysis of membrane phospholipids by cPLA2-α is still an open question.

ARACHIDONIC ACID ACTIONS AND HOMEOSTASIS IN BRAIN AND ALZHEIMER'S DISEASE

ARA binds and activates several protein kinases such as PKN (Mukai et al., 2003) and PKCξ (Kochs *et al.*, 1993). PKN family is constituted by 3 PKC-homologous serine/protein kinases, the first one, PKNα, being highly expressed in brain (Mukai, 2003). In addition, PKN family members are activated at least *in vitro* by ARA or linoleic acid and by Rho family GTPases that were identified as important regulators of the actin cytoskeleton (Mukai, 2003). In agreement with their relation by Rho GTPases, PKNα is involved in the regulation of neuronal microtubules and phosphorylates Tau (Kawamata *et al.*, 1998). Accumulation of PKNα was found in neurofibrillary tangles (Taniguchi *et al.*, 2001). However, the role of free ARA in putative dysregulation of PKN has not been determined until now in AD or other neurodegenerative pathologies. Excessive striated muscle growth in the young cPLA2-$\alpha^{-/-}$ mice has been associated to the inactivation of PKCξ and the suppression of its repressive effect on the IGF-1 signaling pathways. These results and their conclusion are of interest for AD process since IGF-1 has a positive action in AD and has been tested in mice (Zemva & Schubert, 2013). Activation of PKCξ by excessive intraneuronal concentrations of free ARA would potentially inhibit IGF1 pathway. Increased levels of PKCξ expression have been measured in neuronal membrane fraction of AD TG2576 mice (Rossner *et al.*, 2001) and in a T-lymphocyte subpopulation of severe AD

patients (Miscia *et al.*, 2009). Furthermore, PKCξ contributes to Tau phosphorylation by targeting leucine-rich repeat kinase 2 (LRRK2) whose mutation is the most frequent cause of genetic forms of Parkinson disease (Zach *et al.*, 2010). Besides the protein kinase activation, the other main neuronal process modulated by ARA is the formation of SNARE complex and the resulting exocytosis of neuromediators. ARA induces SNARE formation by interacting with syntaxin-1A and munc18 (Rickman & Davletov, 2005; Latham *et al.*, 2007; Connel *et al.*, 2007). Activation of SNARE and exocytosis can be blocked through the sequestration of ARA by α-synuclein whose mutations are implicated in the genetic forms of Parkinson disease (Darios *et al.*, 2010). Furthermore, syntaxin-1 and munc18 also interact with XL11 and APP to segregate APP in peculiar membrane microdomains which differ from those containing BACE-1 (Sakurai *et al.*, 2008). Phosphorylation of munc18 by cdk5 induces a shift of APP to BACE-1-containing domain (Sakurai *et al.*, 2008). Munc18 can also be phosphorylated by the dual-specificity tyrosine[Y]-phosphorylation-regulated kinase 1A [Dyrk1A] which might be responsible for the early onset of AD in Down's syndrome patients (Park *et al.*, 2012). Whether intraneuronal free ARA could modulate the interaction between syntaxin-1A, Munc18 and APP has not been investigated in these studies. Munc18, XL11 and cdk5 were found upregulated in the temporal and occipital cortex of AD patients but not in TG2576 mice (Jacobs *et al.*, 2006). This upregulation only in humans has been assumed as a compensatory mechanism to maintain synaptic functions and avoid excessive production of Aβ peptide in surviving neurons.

The actions of free ARA (Figure 4) and its cellular fate depend on its uptake by several fatty acid-binding proteins (FABP) which also transport other fatty acids to different organelles and/or enzymes. Three FABPs have been characterized in brain until now (Liu *et al.*, 2010). FAPB7 (or brain FABP, B-FABP) contributes to glioma infiltration in presence of high ARA/DHA ratios (Mita *et al.*, 2010). FABP5 (or epidermal FABP, E-FABP) favors neurite growth in neuroendocrine PC12 cells in presence of neuronal growth factor and ARA, DHA or eicosapentaenoic acid (Liu *et al.*, 2008). Deletion of FABP3 (or heart FAPB, H-FABP) gene reduces ARA distribution by 25% in phosphatidylinositol, which is noteworthy since phosphatidylinositol contains high amounts of ARA in brain as well as in other tissues (Tanaka *et al.*, 2003) and ARA turnover is especially high in this phospholipid group in the neuronal growth cone (Negre-Aminou & Pfenninger, 1993). Activation of fatty acid by various acyl-CoA synthetases (ACSLs) is critical for their incorporation in membrane phospholipids and

triglycerides. Among the various ACSLs, ACSL4 specifically uses ARA as substrate and was originally characterized in steroidogenic tissues (Kang *et al.*, 1997). Thereafter, its mutation was associated to X-linked mental retardation (Piccini *et al.*, 1997; Meloni *et al.*, 2009). Two isoforms of ACSL4 are expressed in neuronal cells including a neuron-specific one with an additional 41 amino-acid N-terminal domain (Meloni *et al.*, 2009: Cho *et al.*, 2012). ACSL-4 expression increases during neuronal cell differentiation and neurite growth (Meloni *et al.*, 2009: Cho *et al.*, 2012). The reduction of its expression by silencer RNAs alters the dendritic spine architecture in rat hippocampal neurons (Meloni *et al.*, 2009). Mutations of its analog in drosophila alter the axonal transport of vesicles and synaptic development (Liu *et al.*, 2011). These results suggest that ACSL4 could play a role in AD in which synaptic dysfunctions are the first alterations. Furthermore, overexpression of ACSL4 reduced basal and IL-1β-induced PGE2 secretion in vascular smooth muscle cells (Golej *et al.*, 2011). Conversely, acute pharmaceutical inhibition of ACSL4 by triacsin C enhanced PGE2 release in this study without upregulating COX-2 expression. But the authors also observed that long-term reduction of ACSL4 expression by stably expressed siRNA also reduces PGE2 production and underline that this sustained downregulation affects cell growth in vascular smooth muscle cells like in tumor cells. ACSL4 expression has not been reported in glial cells, but the mutation of it drosophila paralog drastically reduces the number of glial and neuronal cells in the drosophila brain development (Zhang *et al.*, 2009). The expression of ACSL4 in human glial cells could limit prostaglandin production and have some influence on the extension of neuroinflammation in AD. The other main result reported in the role of ACSL4 in vascular smooth muscle cells is that overexpression of ACSL4 increased ARA incorporation by 3-fold into phosphatidylethanolamine and phosphatidylinositol, and by 1.5-fold in triacylglycerol of vascular smooth muscle cells. By expressing several ACSL4 isoforms in CHO cells, Küch et al., (2013) showed that exogenous ARA is preferentially channeled towards phosphatidylinositol by ACSL4. Moreover, ARA incorporation into phosphatidylinositol is selectively catalyzed by lysophosphatidylinositol acyltransferase 1 (LPIAT1, also known as MBOAT7) which is a putative ACSL4 partner. Reduction of its expression drastically affects neurite growth and LPIAT1$^{-/-}$ mice display cortex and hippocampus atrophy which leads to their death within a month (Lee *et al.*, 2012).

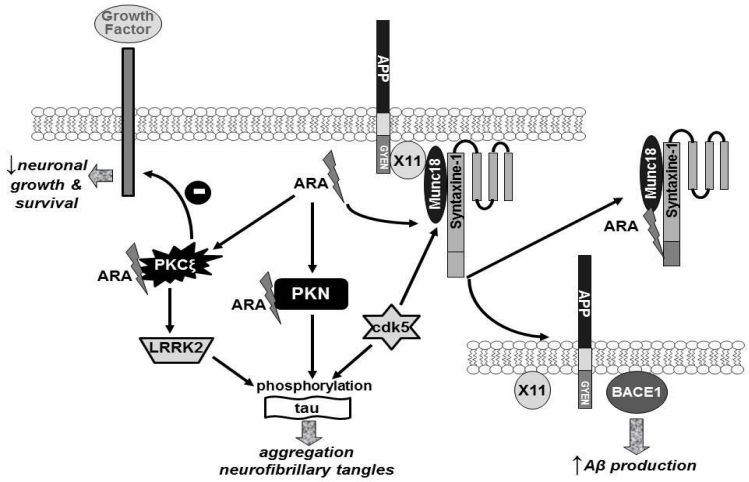

Figure 4. Putative targets and modes of action of free arachidonic acid in AD.

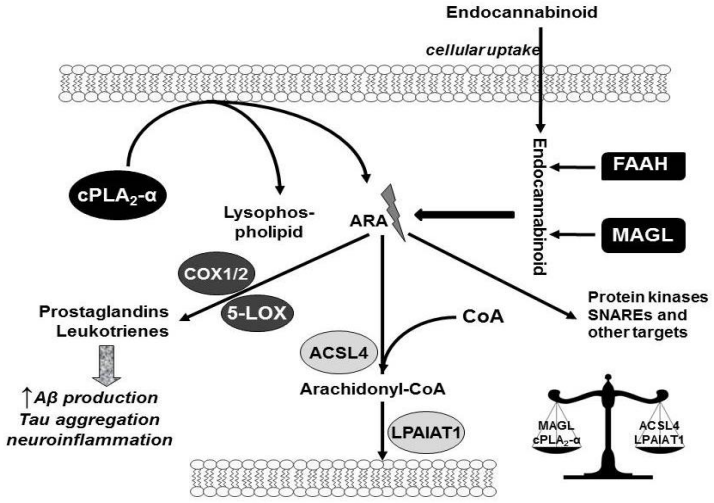

Figure 5. Balance between the enzymes which release or incorporate arachidonic acid. Free arachidonic acid levels in neuronal cells are increased through the degradation of endocannabinoids by MAGL/FAAH and the release of arachidonic acid from membrane phospholipids by $cPLA_2$-α. On the contrary, ACSL4 and LPAIAT1 or other lysophospholipids transferases decrease these levels by favoring incorporation of arachidonic acid into membrane phospholipids. Therapeutic or preventive strategies against AD could target this balance by stimulating the activities of ACSL4 and lysophospholipid acyltransferases.

Taken together, these results indicate that ACSL4 and LPAIAT1 combined action can limit free ARA levels resulting from the $cPLA_2$-α or MAGL activation and limit its conversion into neurotoxic eicosanoids (Figure 5). The other ways to limit release of free ARA into neuronal cells would be to avoid excessive concentrations of ARA in brain membrane phospholipids. Polyunsaturated fatty acids, mainly ARA and DHA, are either synthetized by astrocytes in brain, either provided by food and liver synthesis. Diets in Western countries incorporated more and more ω-6 fatty acids in the last decades. Epidemiological studies evaluate that ω-6/ω-3 precursor ratio increased from 6 to 10 and more in USA between the beginning and the end of the last century (Blasbalg *et al.*, 2011). The situation should be close in the other high-income countries and probably in many mid or low-income counties where obesity prevalence increases. A tremendous number of studies has been devoted to the influence of deficiencies or supplementations of dietary ω-3 fatty acids on cognitive functions in humans and animals (for review, see Luchtman & Song, 2013). But a little number of works studies the influence of excessive amount of ARA or ω-6 precursors in food. High amounts of linoleic and ARA in food to piglets reduce their cerebral accretion of DHA and compromise neurite growth (Novak *et al.*, 2008). A diet enriched with 2% ARA given between 3 to 24 weeks of age was associated with an increase in number and size of amyloid plaques in cortex and hippocampus of double mutant APP mice as compared to the standard diet-fed animals (Amtul *et al.*, 2012). Cognitive abilities in a spatial learning task were compared in young and old rats in association with the fatty acid contents of various brain regions (Yetimler *et al.*, 2012). This study confirmed low cognitive abilities in old rats compared to the young group. But old rats displayed large interindividual variations and good/poor learners could be distinguished in each group. No correlation was observed in both group between DHA brain and cognitive abilities. Young good learners had lower ARA amounts in hippocampus than young poor learners, but no difference was observed in the old group. The results of these last two groups argue in favor of the benefit of low amounts of ARA in food. But positive effects of ARA supplementation have been reported in the alleviation of age-related deficiencies of various brain functions in animals (Okaichi *et al.*, 2005; Fukaya *et al.*, 2007). Therefore, additional studies are necessary to evaluate the optimal dietary amounts of ARA and ω-6 precursors in humans at various ages to prevent and decrease the occurrence of the age-related neurodegenerative disease such as AD.

CONCLUSION

ARA is one of the two main PUFAs in brain with DHA. ARA through direct effects or upon conversion into various eicosanoids can favor AD process and progression, not only by increasing neuroinflammation but also by enhancing synaptic and neuronal dysfunctions. It is therefore a critical actor in the vicious cycle of AD which associates synaptic alterations, neuronal degeneration, glial cell proliferation, synthesis of neuroinflammatory mediators. Free intracellular ARA is released from phospholipids or from endocannabinoid by $cPLA_2$-α or MAGL, respectively. It can be converted into prostaglandins by cyclooxygenases and prostaglandin synthases or to leukotrienes or HETE by 5-LOX and other lipoxygenases. Taking into account this complex network of reactions, therapeutic strategies targeting ARA roles should combine several inhibitors or use pluripotent molecules. The finding of such molecules and new clinical studies are necessary to determine whether this strategy can provide efficient solutions in the fight against AD. Besides the inhibition of $cPLA_2$-α or MAGL, favoring the re-incorporation of free ARA into membrane phospholipids would be an alternative way to limit its intracellular levels and to slow its conversion into eicosanoids. The interplay of ACSL4 and LPAIAT1 or other lysophospholipid acyltranferases constitutes a new putative target which should be further investigated on AD cellular and animal models. But, adequate release of ARA at the right place and at the right moment is probably necessary to allow neuronal functions and synaptic plasticity. These studies will also provide new essential informations about the regulation of ARA cellular homeostasis. Nutritional studies combined to experiments on cellular models are also required to improve the knowledge about the role of ARA in aging and AD. The determination of the adequate dietary ARA and ω-6 intake at different ages of life will be helpful to design preventive strategies against pathological aging.

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