Revisiting the role of acetylcholinesterase in Alzheimer’s disease: cross-talk with P-tau and β-amyloid

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INTRODUCTION
Alzheimer’s disease is the most common cause of dementia among the elderly and is characterized by loss of memory and other cognitive functions. The major pathological hallmarks include extensive synaptic and neuronal loss, astrogliosis, and accumulation of proteinaceous deposits. The AD brain is characterized by the presence of β-amyloid plaques and neurofibrillary tangles (NFT), which are the hallmark pathological features (for a review see Blennow et al., 2006). β-amyloid plaques are extracellular deposits of which the major component is the β-amyloid protein (Aβ), a small polypeptide generated by processing of a much larger transmembrane β-amyloid precursor protein (APP; Masters et al., 1985; Kang et al., 1987) through the successive action of proteolytic enzymes known as secretases (for a recent review see Zhang et al., 2011). The intracellular NFT are composed of paired helical filaments of the microtubule-associated protein tau, which is abnormally hyperphosphorylated (P-tau; Grundke-Iqbal et al., 1986). Today, the focus on research has moved away from the proteinaceous deposits toward studies on the role of the triggering effectors, soluble oligomeric Aβ, and P-tau. Accordingly, much research is devoted to understanding how Aβ and P-tau lead to the toxic events associated with AD, how they cause changes in the expression of other key brain proteins and ultimately how they cause neurodegeneration. However, it is also crucial to decipher how both Aβ and P-tau interact in order to reach a better understanding of the mechanism of neurotoxicity and to achieve an effective therapy.

As extensively reviewed in this special issue, acetylcholinesterase (AChE) is a key enzyme in the cholinergic nervous system. During the progression of AD, many different types of neurons deteriorate, although there is a profound loss of forebrain cholinergic neurons, which is accompanied by a progressive decline in acetylcholine (Davies and Maloney, 1976; Perry et al., 1977). Both the acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT), as well as the acetylcholine-hydrolyzing enzyme, AChE are affected. Therapies designed to reverse the cholinergic deficit are in large measure based on the importance of cholinergic function in cognition. In spite the overall decrease in the activity of AChE in the AD brain, current AD therapy is mostly based on inhibitors of AChE (AChE-I), which enhance cholinergic transmission, but which have modest and transient therapeutic effects (Giacobini, 2002; Kadaszkieiwicz et al., 2005). As a consequence of its role as a target for AD therapy, AChE is one of the most studied proteins in the Alzheimer’s field, with about 1500 manuscripts indexed into the PubMed; the vast majority of reports in the field relate with treatment strategies associated with the use of AChE-I.

It has been well known for almost 50 years that the distribution of AChE molecular forms is particularly affected in the AD brain, but the physiopathological significance and subsequent implications of these intriguing changes in AChE species remain unknown. An increase in AChE levels around amyloid plaques and NFT is a common feature of AD neuropathology, and although the significance of this increase remains to be determined. In another way, up-regulation of AChE activity following long-term AChE-I therapy has been reported in a number of studies during the last decade. All these abnormalities in AChE expression patterns, as well AChE up-regulation in reaction to chronic inhibition, may are related with the limited efficiency and persistence of AChE-I.

In summary, after decades of study and hundreds of reports, AChE remains of considerable interest into the AD field. The description of changes in AChE levels and forms in the AD brain has merit extensive revision (see for example Younkin et al., 1986; Mesulam and Geula, 1990; Massoulié et al., 1993; Layer, 1995; Small et al., 1996; Kása et al., 1997; Grisaru et al., 1999; Talesa, 2001; Rees and Brimijoin, 2003; Ballard et al., 2005; Silman and Sussman, 2005;
Acetylcholinesterase can exist in several different molecular forms, which have specific patterns of expression in different cell types (for a review see Massoulié, 2002). Moreover, the specific subcellular distribution of each species of AChE probably reflects different physiological functions for each form. Indeed, a large number of studies suggest that AChE could have novel functions unrelated to cholinergic neurotransmission (for review see Massoulié et al., 1993; Layer, 1995; Small et al., 1996; Soreq and Seidman, 2001). In this regard, it is important to note that AChE is present in both cholinergic and non-cholinergic brain areas, where the functional significance of non-cholinergic AChE remains unknown. We particularly refer to the work of Mesulam (2004) for a detailed view of the distribution of AChE in the non-pathological and AD brain. Even in cholinergic areas, it has been suggested that the reduction of AChE activity in the AD brain is not due to cholinergic depletion alone, as the density of AChE-rich (cholinergic) fibers decreased in cortical areas of the AD patients but was not correlated with the number of AChE-rich neurons (Jucker et al., 1992). Therefore, it is important to note that an alteration in AChE levels may not reflect a change in cholinergic neurotransmission.

Not all molecular forms of AChE are equally affected in the AD brain. Studies using sucrose gradient centrifugation have revealed two major forms of AChE in the mammal brain, tetrameric and monomeric species (Figure 1; reviewed in Massoulié et al., 1993). The major forms in the non-AD adult brain are tetramers (G4) that are anchored in the cell membrane of neurons. These tetramers probably constitute the true cholinergic species. Other minor species are monomers (G1) and dimers (G2) that cannot be completely separated from each other by sucrose gradient centrifugation. Regional variations in the AChE molecular form ratio G4/G1 usually been studied in relation to neurochemical and neuroanatomical, particularly cholinergic, features of the brain (Atack et al., 1986). However, in the AD brain, there is a selective loss in the G4 form, while the lighter species are preserved (Atack et al., 1983; Fishman et al., 1986) or even increased in severely affected cases of AD (Figure 1; see also Arendt et al., 1992; Sáez-Valero et al., 1999). Similarly, changes in AChE molecular forms in cerebrospinal fluid (CSF) reflect changes in the brain (Sáez-Valero et al., 1999, 2000a). Light AChE species, which represent the major forms in plasma, are also increased in the AD plasma (García-Ayllón et al., 2010). In agreement with human studies, AChE monomeric species are also increased in brain of the APPC100 transgenic mouse which express human Aβ (Figure 2; see also Sberna et al., 1998; Fodero et al., 2002; Sivervyra et al., 2011a) and in rats given intracerebral Aβ (Sáez-Valero et al., 2002). Different reports have corroborated the possibility that Aβ might influence AChE (Sberna et al., 1997; Hu et al., 2003; Melo et al., 2003). So far, the significance of this particular increase in monomeric AChE around plaques and in Aβ models is unclear.

The purpose of this article is to review changes in AChE expression in the AD brain, but with a particular emphasis on the role of these changes in the pathophysiology of AD. In addition, we summarize our recent findings about the cross-talk between AChE and Aβ, and also between AChE and P-tau. The possibility that Aβ and P-tau interact through AChE is considered.

**ALTERED AChE MOLECULAR FORM PATTERN IN AD**

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**SIGNIFICANCE OF INCREASED MONOMERIC AChE IN AD**

Light forms of AChE in the brain have been generally considered as biosynthetic precursors of the G4 forms due to the fact that oligomeric forms of AChE are assembled from monomeric precursors (Brockman et al., 1986) and that once assembled these forms do not interconvert (Rotundo, 1988). Although it is also possible that a pool of monomers represents a separate pool with a different physiological role than that of the major cholinergic G4 form (Small et al., 1996; Grisaru et al., 1999). Because G1 species...
are precursors of higher molecular weight species, and as a consequence G4 is always present in association with the G1 form, it is difficult to identify a specific functional pool of G1 species which is distinct from the precursor pool. Interestingly, in this context, the prevalence of lighter AChE forms in AD brain resembles an embryonic pattern of expression (Arendt et al., 1992). In the human embryonic brain, the major form of AChE is a monomeric species (Muller et al., 1985). Indeed, the expression pattern of AChE forms within the embryonic brain depends on the development stage (Zakut et al., 1985; Perry et al., 1986). The G4 species increase during human brain maturation and become the most abundant before 11 weeks of gestation (Muller et al., 1985). In rodents, the situation is similar with light AChE being the prevalent embryonic forms (Rieger and Vigny, 1976; Sung and Ruff, 1983). However, there is a shift in the molecular forms that occurs post-natally (Muller et al., 1985). The G1 AChE form is the more abundant during all gestational periods (Figure 3). Interestingly, the distribution in molecular form of the structurally related enzyme butyrylcholinesterase (BuChE) does not change significantly either during fetal development or in AD (Atack et al., 1987). The physiological significance of the early and sustained expression of embryonic G1 AChE, where a role in neurotransmission is not clear, is unknown. However, it has been suggested that AChE has roles in development, such as neuronal differentiation, regulation of cell growth, or cell adhesion. These novel functions may depend on protein-protein interactions rather than the enzyme’s catalytic activity (Brimijoin and Koenigsberger, 1999; Paraoanu et al., 2006). On this basis, it has been suggested that AChE in AD may be similar to embryonic AChE, and that it may reflect the activation of a neuronal repair in the AD brain (Layer, 1995).

Therefore, we can speculate that G1 AChE has a non-cholinergic role during brain development, and that this role may be unrelated to the enzyme’s catalytic properties. In this context, it is assumed that all AChE forms possessed similar catalytic properties, which is probably true when oligomeric and monomeric precursors are compared. Nevertheless, it has been demonstrated subtle differences in sensitivity to inhibitors and in kinetic properties exist between tetrameric and monomeric AChE species (Ogane et al., 1992; Rakonczay, 2003). More interestingly, embryonic G4 AChE shares similar biochemical and kinetic properties with the adult enzyme, but embryonic G1 AChE differs in its kinetic properties and in its affinity for several AChE-I from the adult G1 form (Moreno et al., 1998). In this context, AChE activity present in the AD brain associated with plaques and NFT displays particular enzymatic properties and sensitivity to inhibitors (Geula and Mesulam, 1989; Wright et al., 1993).

Therefore, if we accept the possibility that embryonic AChE may possess a function independent of its catalytic capacities, the large pool of catalytically inactive AChE protein should be considered. The basis for the presence of an embryonic AChE species different from that of the adult, is unknown, but several questions arise regarding the use of AChE-I in AD therapy.

The existence of an unexpectedly large pool of inactive AChE has been demonstrated in brain (Chatel et al., 1993) and other tissues (Stieger et al., 1987; Rotundo, 1988; García-Ayllón et al., 2006), and suggested in CSF (García-Ayllón et al., 2007). This inactive pool may have non-classical functions, as it has been demonstrated that transgenic over-expression of enzymatically inactive AChE can influence neurodevelopment (Dori et al., 2005). The inactive AChE fraction is proportionally more abundant in embryonic than in adult tissues (Masoulié et al., 1993). Because routinely AChE levels are estimated enzymatically using various modifications of the Ellman et al. (1961) method, and are not normally estimated immunologically, little information is available on this inactive pool of AChE in pathological and non-pathological conditions. A significant decrease of both AChE activity and immunoreactivity has been observed using enzyme-linked immunosorbent assay of AD brain (Hammond and Brimijoin, 1988). However, after decades of studying AChE in the AD brain further research is still necessary in order to determine the AChE protein content and its relationship with altered AChE activity levels. Whether non-catalytic AChE in brain has physiological significance, and how it is affected during pathology and treatment, are issues that warrant further study.

This complex scenario of multiple molecular forms is brought about, at least in part, by the existence of alternative splicing of

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**FIGURE 3** | Monomeric AChE molecular forms are the predominant species during embryonic brain development. AChE activity was extracted from rat (Sprague-Dawley) cerebral cortex at embryonic day 12 (E12), 14 (E14), 16 (E16), and 18 (E18) and at 3 months of age (Adult), and AChE forms were separated by sucrose density gradient (see Figure 1). At early embryonic stage only light monomeric G1 AChE is present and levels are maintained (no change; n.ch.) until E15–16, an increase in G4 AChE parallels emergence of G4, AChE tetramers, the brain cholinergic species. The G1 species increase in activity during brain maturation becoming the major molecular forms during post-natal periods (N.B. difference of scales between embryonic and adult stage).
the single AChE gene, generating different AChE variants, with potential different gene regulation (Grisaru et al., 1999; see also Figure 4). Alternative 3′ RNA splicing generates different polypeptide encoding transcripts called “tailed” or T, “hydrophobic” or H, and “readthrough” or R-transcripts (Massoulé et al., 1993; Taylor and Radic, 1994; Grisaru et al., 1999), with the same catalytic domain and distinct C-terminal peptides that determine the ability of the molecule to form oligomers. In the mammalian brain, the T-transcript is the major form and encodes subunits which produce monomers, dimers, and tetrameric forms; whereas the R-transcript, which is normally present at low levels (Kaufer et al., 1998; Perrier et al., 2005), encodes a soluble monomeric form (Sternfeld et al., 2000; Figure 4). In addition to the 3′ alternatively spliced species of AChE, the 5′ end is also subject to intricate regulation (Mesherer et al., 2004) generating AChE variants that have extended N-terminals; thus, within the brain N-AChE-T and N-AChE-R variants may occur in parallel with AChE-T and AChE-R. The AChE-T and AChE-R monomers or their N-extended variants cannot be distinguished by molecular weight. Whether the increase in monomeric AChE in the AD brain is related to increases in the T or R variants is still an open question. Anyhow, in this context has been suggested that the AChE-R can compete with the main brain AChE-T protein and suppress the formation of insoluble Aβ oligomers (Berson et al., 2008; see below). An increased N-AChE-T expression has been also postulated in the AD brain associated with disease progression, including apoptotic cell death (Toiber et al., 2008). In summary, Soreq and Seidman (2001) have presented evidence that N- and C-terminally modified AChE variants, all of which have similar enzymatic activities, can display distinct and in certain cases inverse functions (reviewed in Greenberg et al., 2010). The use of AChE-I which does not distinguish between AChE variants should interfere in all processes indiscriminately.

The central question is whether the changes in the distribution of AChE molecular forms in the AD brain have any physiopathological consequences. As stated previously, gross sedimentation analysis cannot distinguish between monomeric isoforms that are synthesized to be assembled in oligomers, or arise as degradation products, and those specific monomeric species which may have specific functions. Therefore, in previous studies we have further characterized the increase in monomeric AChE associated to Alzheimer’s and to Aβ by characterizing its glycosylation pattern by lectin binding analysis, based on the assumption that different functional pools of AChE may have different glycosylation patterns. Correct glycosylation determines the adequate intracellular trafficking, folding, assembly, and final localization of glycoproteins. Thus different forms (glycosylated variants or glycoforms) of the same protein should differ in glycosylation in order to achieve a different oligomerization state, subcellular localization, protein–protein interaction affinity, or a different physiological function. Indeed, for a particular glycoprotein, the abundance of single glycoforms should closely correlate to each other and be regulated within narrow limits. By exploiting the ability of lectins to bind diverse carbohydrate moieties with high specificity (Sharon and Lis, 2004), we have demonstrated that the glycosylation of AChE is altered in the AD brain, postmortem, and lumbar CSF (Sáez-Valero et al., 1997, 1999, 2000a). Tetrameric G4 and light G1 have different glycosylation patterns (Sáez-Valero et al., 1999), thus depletion specific loss of the tetrameric form in the AD brain may be responsible, in part, for this change. Changes in AChE glycosylation were also characterized in Aβ transgenic mice models displaying increases in monomeric AChE (Sberna et al., 1998; Fodero et al., 2002). However, we further demonstrated that when the light AChE species from AD and non-demented brain are isolated, G1 species present in AD brain displayed different affinities for lectins and for conformational anti-AChE antibodies, compared with isoforms from control brains (Sáez-Valero et al., 2000b). These changes indicate that the minor subset of G1 AChE, whose contribution is increased in AD brain, correspond to isoforms (glycoforms) either not present, or poorly present in adult human brain in non-disease conditions. The physiological relevance of the increase in this minor G1 form for AD pathogenesis is unclear.

Alterations in the glycosylation state of other glycoproteins have been reported in AD tissue (Guevara et al., 1998; Fodero et al., 2001; Kanninen et al., 2004; Sihlbom et al., 2008). Pathological impairment in the broader protein glycosylation machinery could significantly compromise the processing of many glycoproteins, thereby resulting in loss of physiological function of many of these proteins. Abnormal incorporation of carbohydrate moieties in AChE subunits can compromise its functional role and/or oligomerization. In this context, altered AChE glycosylation has

![Human AChE gene](image-url)

**FIGURE 4** | Diagram of the human AChE gene structure, its alternative splicing and generation of variants and molecular forms of AChE in brain. Gene structure showing the splicing pattern of the six major exons. The catalytic core of human AChE is encoded by three exons and additional exons encode the variant-specific carboxy-terminal sequences. In the normal human brain, much more AChE-T than AChE-R mRNA is produced. However, under stress AChE-R expression is increased. To date, no expression of AChE-H has been described in the brain (see Grisaru et al., 1999 for more details). The R-transcript encodes a soluble monomeric form, while the T-transcript encodes a wide variety of monomeric (G1) and oligomeric (G4, G1, and G2) molecular forms (Massoulé et al., 1993). AChE molecular forms can vary in their solubility characteristics (non-amphiphilic: “−”, amphiphilic: “+”). The main amphiphilic G4 form in brain is anchored to the membrane by a “proline-rich membrane anchor” PRiMA subunit (Perrier et al., 2002). Additional complexity can be generated by additional 3′ alternatively splicing which can generate AChE variants with extended N-terminals (not shown in figure; see Mesheror et al., 2004).
been also demonstrated in Creutzfeldt-Jakob disease (Silveyra et al., 2006), a neurodegenerative process where impaired glycosylation machinery is suspected (Rudd et al., 1999). Nonetheless, unlike AD, in Creutzfeldt-Jakob disease, altered AChE glycosylation is not caused by changes in the proportion of any particular molecular form (Silveyra et al., 2006). Therefore, we favor the hypothesis that in Creutzfeldt-Jakob disease change in AChE glycosylation is the consequence of perturbed glycosylation machinery, whereas the altered glycosylation pattern of light AChE species in AD reflects an imbalance of protein glycoforms resulting from changes in AChE variants related or not to differentiation state. This is an issue that requires further study.

P-TAU INCREASES AChE, DIFFERENCES FROM A

It has been noted that abnormal AChE expression in the AD brain occurs in association with the two hallmark features of the AD pathology, the amyloid plaques and the NFT (Mesulam and Morán, 1987; Ulrich et al., 1990). As mentioned previously, Aβ peptides influence AChE levels, thus Aβ may be responsible for increased AChE around plaques. However, the increase in AChE associated with NFT has remained largely unexplored. Recently, we showed for the first time that P-tau can trigger an increase in AChE expression (Silveyra et al., 2011a). In vivo over-expression of P-tau in transgenic mice (Tg VLW mice) expressing human tau mutations causing frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) led to an increase in the activity of the T-transcript of AChE (Silveyra et al., 2006). Therefore, we favor the hypothesis that in Creutzfeldt-Jakob disease change in AChE glycosylation is the consequence of perturbed glycosylation machinery, whereas the altered glycosylation pattern of light AChE species in AD reflects an imbalance of protein glycoforms resulting from changes in AChE variants related or not to differentiation state. This is an issue that requires further study.

DOWNSTREAM CONSEQUENCES OF CHANGES IN AChE LEVELS IN AD BRAIN

Notwithstanding the overall loss of total AChE activity in the AD brain, AChE is consistently increased in regions around amyloid plaques and NFT at all stages of the disease, including some of the earliest stages (Perry et al., 1980; Mesulam and Morán, 1987; Ulrich et al., 1990). Extensive studies by Inestrosa et al. (1996) suggest that AChE may directly interact with Aβ in a manner that increases deposition of Aβ to form plaques. Studies using double transgenic mice that over-express AChE and Aβ (Rees et al., 2003) support these observations and they suggest that AChE may play a role in pathogenesis of AD.

Our own recent work also indicate that AChE can modulate APP processing and Aβ production. Aβ is produced through the successive action of two proteolytic enzymes, β-secretase and γ-secretase on APP (Zhang et al., 2011). The active proteolytic component of the γ-secretase complex is presenilin 1 (PS1; Süh and Checler, 2002). Mutations in PS1 cause early-onset AD with an accelerated rate of Aβ deposition (St George-Hyslop, 2000), thus proteins that interact with PS1 are of major functional importance. We have previously demonstrated an interaction between AChE and PS1 by reciprocal co-immunoprecipitation (Silveyra et al., 2008). Recently, we demonstrated that AChE can influence PS1 levels by showing that AChE over-expression increases PS1 levels, while AChE knock-down with siRNA leads decrease PS1 in transfected cells (Silveyra et al., 2011b). Perhaps the most significant conclusion from our recent study is the potential participation of AChE in a degenerative cycle that enhances amyloidogenic APP processing. We can presume that several degenerative cycles, participating as interactive systems within a larger vicious cycle, accelerate the development of AD. Hence, in AD it is possible that Aβ can induce a feedback loop leading to amyloidogenic APP processing (Cribbs et al., 1995; White et al., 2003). A vicious cycle of Aβ generation potentially could involve PS1; recent evidence indicates that Aβ42 can induce an increase in PS1 levels in cultured neurons creating a toxic loop (Matrone et al., 2008). Using different experimental conditions, we have confirmed that both Aβ42 and P-tau trigger an increase in AChE, which can in turn influence PS1 and thereby modulate Aβ production (Figure 6). We have found that Aβ42-induced PS1 increase can be prevented by pre-treatment of SH-SY5Y cells with siRNA AChE (Silveyra et al., 2011b). The possibility that different effects may be obtained with different AChE species and variants (tetrameric versus monomeric molecular form, or T-variant versus R-variant or N-extended variant), requires further study. In this context, recent evidence indicates a
specific role inducing GSK-3β activation and tau hyperphosphorylation for the alternative N-terminally extended T-AChE variant (Toiber et al., 2008), variant up-regulated by stressors inducing protein misfolding and calcium imbalances, both characteristic of AD.

It appears likely, therefore, that several vicious cycles trigger by Aβ and P-tau involve the potential participation of AChE. The significance of these theoretical considerations to the clinical and neuropathological course of AD remains to be demonstrated.

**EFFECTS OF AChE-I**

Therapies designed to reverse the cholinergic deficit are in large measure based on the importance of cholinergic function in cognition. Indeed, AChE-Is have proven to be modestly efficacious in treating the cognitive and functional symptoms of AD. In addition disease-modifying effects of AChE inhibition has been also considered (Giacobini, 2002). However, the modest clinical efficacy of AChE-Is has not discouraged the development of new AChE-Is, and particularly the so called dual binding site AChE-Is (Muñoz-Torrero, 2008; Pepeu and Giovannini, 2009), which are both inhibitors of AChE and also of amyloid plaque formation. This latter effect is based on the fact that AChE-binding molecules may, in addition to their effect on enzyme activity, block the effect of AChE on Aβ fibrillogenesis by interaction throughout the peripheral anionic site of the enzyme (Inestrosa et al., 1996). Besides the design of new inhibitors with the capacity to block catalytic and peripheral anionic sites of AChE, there is evidence that inhibitors may also influence APP processing. AChE-Is have been shown to alter APP expression and metabolism in cellular (Lahiri et al., 1994; Pakaski et al., 2001; Peng et al., 2006) and animal models (Mori et al., 1995; Zimmermann et al., 2004; Dong et al., 2009), as well in AD-treated patients (Clarke et al., 2001; Basun et al., 2002; Zimmermann et al., 2005). The modulatory effects of AChE-I on APP metabolism have been attributed to their effect on ADAM10/α-secretase (Zimmermann et al., 2004; Peng et al., 2006), BACE1/β-secretase (Lahiri et al., 2007; Fu et al., 2008; Li et al., 2010), and on PS1/γ-secretase (Silveyra et al., 2011b). The disparity between the effects of AChE-Is and cholinergic agonists on secretase activity would suggest that AChE is not exerting its modulatory action on PS1 via a cholinergic mechanism (Zimmermann et al., 2004; Silveyra et al., 2011b). However, effects on APP processing by cholinergic agonists have been also demonstrated (Nitsch et al., 1992; Rossner et al., 1998; Davis et al., 2010). The mechanisms by which AChE-Is influence APP processing remain unclear, but it is suggested that regulation of APP processing by AChE-Is may involve multiple mechanisms, including cholinergic and non-cholinergic actions, some independent of their anti-cholinesterase (catalytic) activities (Lahiri et al., 1997).

The therapeutic effect of current AChE-Is is both modest and transient. Current AChE-I treatment results are disappointing both because of their poor efficacy and tolerability. Interestingly, these drugs have a limited duration of cognitive benefit. The effects of these drugs on APP processing also fail to be maintained over the long-term in Alzheimer’s patients (Basun et al., 2002).

The transience of the response to AChE-I could be associated with AChE up-regulation in reaction to chronic inhibition (Chiappa et al., 1995; Kauf et al., 1998). Indeed, increases in CSF AChE have been reported after AChE-I treatment (Davidsson et al., 2001; Darreh-Shori et al., 2006; García-Ayllón et al., 2007; Parnetti et al., 2011). The varying responses of different AChE species to AChE-I treatment were the light AChE species seem not subject to AChE-I induced up-regulation, suggest different modes of regulation and should be also considered (García-Ayllón et al., 2007). Interestingly, we found that the effect of AChE-I treatment on PS1 levels was also not sustained, and that the lack of effect on PS1 was associated with up-regulation of AChE (Silveyra et al., 2011b). Our data suggest that sustained AChE inhibition cannot be effective when the expression of AChE is up-regulated and that this undesired effect needs to be addressed to develop more effective therapies based on AChE-I.

**SUMMARY AND CONCLUSION**

In summary, AChE species differ in their responses to disease and their interactions with β-amyloid and P-tau. The important question about the nature of the alternative functions of AChE, their association with different AChE species and variants, and their role in AD pathogenesis and therapy needs to be examined further. Recent evidence also suggests the potential participation of AChE in vicious cycles involving Aβ and P-tau. Elucidation of the mechanisms involved in these changes will be useful for understanding the physiological and pathological relevance of altered AChE expression in the AD brain and AChE-I pharmacological intervention. The chronic increases in AChE activity during AChE-I treatment may cause the therapeutic value of AChE-I to be limited and temporary and needs to be addressed in order to improve therapy.

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