Targeting acetylcholinesterase and butyrylcholinesterase in dementia

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Abstract

The cholinesterase inhibitors (ChE-Is) attenuate the cholinergic deficit underlying the cognitive and neuropsychiatric dysfunctions in patients with AD. Inhibition of brain acetylcholinesterase (AChE) has been the major therapeutic target of ChE-I treatment strategies for Alzheimer’s disease (AD). AChE-positive neurons project diffusely to the cortex, modulating cortical processing and responses to new and relevant stimuli. Butyrylcholinesterase (BuChE)-positive neurons project specifically to the frontal cortex, and may have roles in attention, executive function, emotional memory and behaviour. Furthermore, BuChE activity progressively increases as the severity of dementia advances, while AChE activity declines. Therefore, inhibition of BuChE may provide additional benefits. The two cholinesterase (ChE) enzymes that metabolize acetylcholine (ACh) differ significantly in substrate specificity, enzyme kinetics, expression and activity in different brain regions, and complexity of gene regulation. In addition, recent evidence suggests that AChE and BuChE may have roles beyond ‘classical’ co-regulatory esterase functions in terminating ACh-mediated neurotransmission. ‘Non-classical’ roles in modulating the activity of other proteins, regional cerebral blood flow, tau phosphorylation, and the amyloid cascade may affect rates of AD progression. If these additional mechanisms are demonstrated to underlie clinically meaningful effects, modification of the over-simplistic cholinergic hypothesis in AD that is limited to symptomatic treatment, ignoring the potential of cholinergic therapies to modify the disease process, may be appropriate. The specificity of ChE inhibitory activity, up-regulation of AChE activity and changes in the composition of AChE molecular forms over time, selectivity for AD-relevant ChE molecular forms, brain vs. peripheral selectivity, and pharmacokinetic profile may be important determinants of the acute and long-term efficacy, safety and tolerability profiles of the different ChE-Is. This review focuses on new evidence for the roles of BuChE and AChE in symptom generation and rate of underlying disease progression in dementia, and argues that it may be appropriate to re-evaluate the place of ChE-Is in the treatment of dementia.

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Introduction

Alzheimer’s disease (AD) is characterized by a loss of cholinergic neurons and their cortical projections from the nucleus basalis and associated areas in the basal forebrain. Cholinergic synaptic function appears to be particularly susceptible to beta-amyloid (Aβ) peptide toxicity, and loss of synaptic vesicles on axon terminals may precede cholinergic neuronal loss (Small et al., 2001). Progressive deterioration of the widespread and dense cholinergic innervation of the human cerebral cortex contributes to the salient cognitive and behavioural disturbances in AD, and is associated with decreased levels of the neurotransmitter, acetylcholine (ACh), choline acetyltransferase (ChAT) – the rate-limiting enzyme for ACh synthesis, and acetylcholinesterase (AChE) – the ACh-hydrolysing enzyme. Remarkably, brain levels of another enzyme that hydrolyses ACh, butyrylcholinesterase (BuChE), show progressive and
Enzyme structure and function

At the molecular level, AChE and BuChE share 65% amino-acid sequence homology and are encoded by different genes on human chromosomes 7 (7q22) and 3 (3q26) respectively (Soreq and Zakut, 1993). Both AChE and BuChE have a primarily hydrophobic amino-acid sequence determining the three-dimensional size and shape of their active site gorge. It has been proposed that the efficiency with which AChE and BuChE hydrolyse ACh is dependent on the substrate concentration. AChE has greater catalytic activity at low ACh concentrations, resulting in substrate inhibition at higher doses (Soreq and Zakut, 1993; Taylor and Radic, 1994). However, BuChE is more efficient at high substrate concentrations.

The peripheral anionic site (PAS) of AChE, the subsite outside of the active site gorge, mediates substrate inhibition of AChE (Ordentlich et al., 1995; Radic et al., 1993). Three aromatic amino acids located at the entrance to the active site gorge are the key residues of this subsite. The PAS of BuChE, which is different from that of AChE, has weaker affinity than AChE for typical PAS ligands and mediates substrate activation. The three aromatic residues of the AChE PAS are missing in the PAS of BuChE, which is formed by two amino acid residues (Asp70 and Tyr332) in the human enzyme. A polymorphism of one of the residues forming the PAS of BuChE is found in the ‘atypical’ BuChE variant (Asp70 mutated to Gly70). This mutant binds succinylcholine weakly, may produce prolonged anaesthesia (Neville et al., 1990), and does not show substrate activation (Masson et al., 1997). The differences in the enzyme kinetic properties and locations of brain AChE and BuChE have led to the suggestion that, in the normal brain, AChE is the main enzyme responsible for ACh hydrolysis, while BuChE plays a supportive, functional role.

Molecular forms of ChE

For both AChE and BuChE, a single gene gives rise to different protein products by alternative splicing in the coding region of the original transcript. For both ChEs, all mRNAs that derive from the original transcript contain a core that is common to each and is essential for catalytic activity of the enzyme, but possess dissimilar carboxyl-terminal sequences that account for their different multimerization, attachment to cell membranes, or existence as soluble proteins (Massoulie et al., 1999). This provides a series of diverse but related molecular forms of AChE and BuChE that have similar catalytic properties but different cellular and extracellular distributions and non-catalytic activities.

AChE exists in asymmetric forms (A forms) containing catalytic units attached to a membrane-anchored protein tail, and three globular forms (G forms) containing one, two or four catalytic subunits (monomeric G1, dimeric G2, and tetrameric G4). G1 AChE (usually encoded for by AChE-R mRNA species – see below) exists solely as a soluble entity,
whereas G4 AChE (usually encoded for, as other multimers, by AChE-S mRNA species — see below) exists in both soluble and membrane-bound forms (Massoulie et al., 1999). In the human brain, AChE is present in G1 and G4 forms, the proportions of which vary in different brain regions (Atack et al., 1986). BuChE also exists in G1, G2, and G4 molecular forms but, similar to AChE, G4 is the predominant isoform in the mature brain (Arendt et al., 1992; Darvesh et al., 2003a).

In the brains of patients with AD, the level of the membrane-bound G4 form of AChE is selectively reduced by 90% or more in certain regions, probably due to the loss of presynaptic terminals. However, levels of G1 AChE remain largely unchanged (Siek et al., 1990). It has been suggested that changes in the expression of AChE molecular forms may be a direct consequence of Aβ (Saez-Valero et al., 2002). The G1 form of BuChE shows a 30–60% increase in the AD brain, probably due to an increase in the number of BuChE-positive glia, while the G4 form decreases or remains the same as in the normal brain. An analogous reduction in neuronal AChE and an increase in neuronal BuChE may accompany peripheral neurodegeneration (Moral-Naranjo et al., 2002). The potencies of ChE enzyme inhibition by the various ChE-Is have been shown to be significantly different from brain region to brain region (Zhao and Tang, 2002) with respect to selectivity for different molecular forms (Enz et al., 1993; Rakonczay, 2003), and in AD relative to the non-AD brain (Guillozet et al., 2003). Taken together, these findings suggest that the use of ChE-Is in the treatment of AD should consider both molecular form-specific and region-specific

### Table 1. Overview of pharmacological characteristics of cholinesterase (ChE) inhibitors (adapted from Poirier, 2002)

<table>
<thead>
<tr>
<th></th>
<th>Donepezil</th>
<th>Rivastigmine</th>
<th>Galantamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical class</td>
<td>Piperidine</td>
<td>Carbamate</td>
<td>Tertiary alkaloid</td>
</tr>
<tr>
<td>Inhibition of ChE enzymes</td>
<td>Non-competitive, rapidly reversible (&lt;1 ms) of AChE</td>
<td>Non-competitive, very slowly-reversible (~6–8 h) of both BuChE and AChE</td>
<td>Competitive, rapidly reversible (&lt;1 ms) of AChE</td>
</tr>
<tr>
<td>Site on inhibition of target enzyme</td>
<td>Covers catalytic gorge and peripheral anionic site, binding to choline anionic site</td>
<td>Catalytic binding site</td>
<td>Choline anionic site and catalytic binding site</td>
</tr>
<tr>
<td>Increased activity/levels of target enzymes during long-term treatmenta,b</td>
<td>5–10 mg/d: ~3-fold increase in AChE levels over 6–12 monthsa</td>
<td>3–12 mg/d – sustained decrease in AChE and BuChE activity over 12 monthsb</td>
<td>24–32 mg/d: ~2-fold increase in AChE levels over 6 monthsa</td>
</tr>
<tr>
<td>Brain vs. peripheral selectivity</td>
<td>Uncertain</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>Preferential isoform selectivityc,d,e</td>
<td>None in most studiesc,d, Gl in onee</td>
<td>G1 (or AChE-R)c,d</td>
<td>None</td>
</tr>
<tr>
<td>Allosteric modulation of nicotinic receptorf</td>
<td>No</td>
<td>No</td>
<td>Particular subtypes (e.g. α4/β2) in vitro</td>
</tr>
<tr>
<td>Metabolism</td>
<td>CYP2D6 and 3A4</td>
<td>AChE and BuChE</td>
<td>CYP2D6 and 3A4</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>~96%</td>
<td>~40%</td>
<td>~20%</td>
</tr>
<tr>
<td>Recommended dose</td>
<td>5–10 mg/d, once daily</td>
<td>6–12 mg/d, twice daily with food</td>
<td>16–24 mg/d, twice daily with food</td>
</tr>
<tr>
<td>Formulations</td>
<td>Tablets</td>
<td>Capsules</td>
<td>Tablets</td>
</tr>
<tr>
<td></td>
<td>Oral solution (in development)</td>
<td>Oral solution Transdermal patch (in development)</td>
<td>Oral solution Once daily controlled-release</td>
</tr>
</tbody>
</table>

AChE, Acetylcholinesterase; BuChE, butyrylcholinesterase; nAChR, nicotinic ACh receptor; CYP450, cytochrome P450.

a Davidsson et al. (2001); b Darreh-Shori et al. (2002); c Enz et al. (1993); d Rakonczay (2003); e Zhao and Tang (2002); f Samochoki et al. (2000).
characteristics of AChE and BuChE inhibition (Zhao and Tang, 2002).

**ChEs in pathophysiological stress**

AChE may have a role in the neurobiology of reactions to stress, which may alter the splicing of the AChE gene to yield different transcripts coding for different molecular forms (Nachon et al., 2003; Soreq and Seidman, 2001). Under normal conditions, much more AChE-S [the ‘synaptic’ variant, usually existing in a multimeric (G4), membrane-bound form] is produced compared with AChE-R [the ‘read-through’ variant, usually existing in a soluble, monomeric (G1) form]. However, under conditions of acute stress, such as following head injury (Shohami et al., 2000), anti-AChE intoxication (Shapira et al., 2000), or during psychological stress (Kaufe et al., 1998), expression of the AChE-R form is greatly increased due to alternative mRNA splicing. AChE-R might contribute to the acute attenuation of neurodeterioration (Sternfeld et al., 2000). For example, transgenic animals that are unable to respond to stress with AChE-R overproduction are exceptionally vulnerable to closed-head injury (Shohami et al., 2000).

It is presumed that stressful stimuli induce excitation through ACh release, and that a feedback response, inducing overexpression of AChE, acts to return excessive cholinergic neurotransmission to normal levels (Kaufe et al., 1998). This may be of importance not only for cholinergic neurotransmission, but also for neurotransmission modulated by ACh, such as glutamate-mediated neurotransmission in the hippocampus (Gray et al., 1996), and for actions mediated by the non-cholinergic function of ACh, such as in the regulation of neuronal cytoarchitecture (Mattson, 1988). AChE mediates other stress-related responses such as the myeloid and megakaryocytic expansion that are characteristic of acute and chronic stress responses (Soreq and Glick, 2000), through the released C-terminal peptide of AChE-R (Grisaru et al., 2001). AChE-derived C-terminal peptide fragments may possess distinct structures and biological activities (Bon et al., 2004).

However, such acutely beneficial stress responses may be harmful in the long term. Transgenic mice overexpressing AChE show progressive cognitive impairments and neuropathological markers, including loss of cortical dendrites, reminiscent of AD (Beeri et al., 1997) and neuromotor deficits (Andres et al., 1997). Although absolute levels of AChE appear important in mediating these effects, it is unclear if the ratio of AChE-S/AChE-R is also important. Massive accumulation of both AChE-S and AChE-R in animal models induces erratic behaviour associated with impaired working memory under conditions of mild stress (Cohen et al., 2002), as well as neurodeterioration (Sternfeld et al., 2000). However, AChE-R accumulation alone protected neurons from damage associated with prolonged conflict behaviour (Birikh et al., 2003). Thus, there may be an optimal AChE-R/AChE-S ratio for the maintenance of optimal physiological function, which can rapidly alter in response to stressors in order to maintain homeostasis. However, chronic increases in, and imbalances of, the ratio of molecular forms of AChE appear to result in cholinergically mediated dysfunctions.

**ChE gene regulation: implications for treatment**

Changes in the protein levels of cerebrospinal fluid (CSF) AChE variants may be reliably used as a surrogate marker of changes in the expression of AChE molecular forms in cholinergic neurons in the brain (Chubb et al., 1976; Tomkins et al., 2001). However, whereas the G4 activity in the CSF appears to reflect activity in the brain, the activity of CSF G1 and G2 forms in the CSF appear to be considerably less than in the brain (Darreh-Shori et al., 2004). The ChE-Is appear to reverse the AD-induced decrease over time in AChE levels (Darreh-Shori et al., 2002, 2004; Davidsson et al., 2001). The ability to induce increases in AChE levels varies greatly amongst ChE-Is. Marked elevations in CSF levels and activity of AChE are seen following long-term treatment with tacrine, donepezil and galantamine (Amici et al., 2001; Davidsson et al., 2001; Nordberg et al., 1999; Parnetti et al., 2002) (Table 1). The expression of AChE-R appears to be more sensitive to the effect of treatment or lack of treatment than that of AChE-S (Darreh-Shori et al., 2004). ChE-I therapy may increase the R/S ratio, and the ability to do this may also vary amongst ChE-Is. For example, rivastigmine was shown to induce greater increases in the R/S ratio than tacrine (Darreh-Shori et al., 2004). The R/S ratio has been highly correlated with sustained cognitive improvement over 1 yr of treatment (Darreh-Shori et al., 2004). Increases in total CSF AChE levels have been correlated with cognitive response in AD patients treated with galantamine and donepezil (Davidsson et al., 2001), but changes in the R/S ratio were not assessed. ChE-Is may also differentially affect CSF G2 AChE protein levels. This largely inactive dimmer was reduced in rivastigmine-treated AD patients, but increased over 2-fold in tacrine-treated and untreated patients (Darreh-Shori et al., 2004). Thus, the various ChE-Is may also differentially affect the inter-protein interactions and multimerization of
AChE variants in addition to their differing potentials to induce increases of AChE and changes in the composition of AChE variants.

Increases in AChE levels could have important consequences if the findings in experimental animals are applicable to humans (Grisaru et al., 1999; Soreq and Seidman, 2001). The amelioration of the symptoms of dementia by ChE-Is, if accompanied by significant increases in AChE expression, may be both limited and temporary (Soreq and Glick, 2000). In addition to tolerance to the symptomatic effects of ChE-I treatment, the potential roles for AChE in disease progression (discussed later in this review) may mean that increased AChE levels accelerate the course of the disease. Increases in AChE levels may also explain certain treatment-emergent adverse effects, and the ‘crashes’ or dramatic worsening of symptoms resembling cholinergic delirium that are observed in some patients when chronic donepezil treatment is discontinued (Bhanji and Gauthier, 2003; Grossberg, 2003; Singh and Dudley, 2003).

The increases in AChE during treatment with particular ChE-Is may be due to the up-regulation of AChE gene expression (Kaufer et al., 1999), reflecting feedback mechanisms via muscarinic ACh receptors (Nitsch et al., 1998). Polymerase chain reaction studies to assess mRNA expression are required before it may be definitely stated that increased AChE levels in patients receiving ChE-Is are due to up-regulation of gene expression. Alternatively, the elevation of AChE levels in treated patients may be indicative of improved functioning of cholinergic neurons due to stimulation (Swaab et al., 1994), or increased regional cerebral blood flow (rCBF) (Venneri et al., 2002). Changes in AChE variant composition may reflect modulation of alternative splicing (Meshorer et al., 2002).

Overall increases in AChE or BuChE activity during long-term treatment are not seen with the slowly reversible dual inhibitor rivastigmine (Darreh-Shoreh et al., 2002; Table 1). Rivastigmine binds directly to the catalytic site and forms a relatively stable acyl-enzyme intermediate that does not undergo hydrolysis for many hours (Bar-On et al., 2002). In fact, significant decreases in AChE and BuChE activity are sustained for at least 12 months of rivastigmine treatment (Amici et al., 2001; Darreh-Shori et al., 2002; Parnetti et al., 2002). A regulatory role for BuChE on the expression of AChE has been suggested (Layer et al., 1992). After the in vitro addition of the specific BuChE-inhibitor (iso-OMPA), not only BuChE activity was completely suppressed but significant influence on AChE activity was also detectable. Although the rapidly reversible dual inhibitor tacrine may up-regulate AChE activity, increases in AChE activity do not appear to be as marked as with selective AChE inhibitors (Nordberg et al., 1998, 1999). Increases in BuChE activity are not observed in CSF following long-term treatment with tacrine or rivastigmine. Thus, increased gene expression may be specific to AChE and could reflect different regulatory pathways for the AChE and BuChE genes (Nordberg et al., 2003).

Interestingly, there is evidence that the irreversible AChE and BuChE inhibitor, metrifonate, also may not produce up-regulation of AChE. In contrast to tacrine, metrifonate maintained red blood cell AChE inhibition over 3 months in patients with AD (Cummings et al., 1998; Pettigrew et al., 1998), confirming findings of no apparent AChE up-regulation by metrifonate in animal models (Hinz et al., 1998). Similar to tacrine, donepezil and galantamine are very rapidly reversible inhibitors. Donepezil induces a strong dose-dependent up-regulation of AChE activity, possibly due to its non-competitive inhibitory action. Galantamine, a competitive inhibitor which depends more on the substrate concentration than on the absolute concentration of the drug, induces less marked elevations of AChE activity. These differential findings amongst ChE-Is support the hypothesis that chemical diversity within the same drug class provides varied pharmacological profiles among the individual agents within the class.

Until very recently, the only established function of AChE was termination of cholinergic neurotransmission, and the use of AChE inhibitors for the treatment of the cholinergic imbalances in AD represented a rational approach. AChE inhibition could be evaluated in terms of the efficiency of inhibition of cholinesterase activity, and the consequences of AChE inhibition assessed by observing cholinergically mediated processes such as cognition. The chronic effects of ChE-Is on AChE activity and changes in the composition of AChE variants requires further evaluation, as any potential clinical impact is currently unknown. Investigation may yield information referring to treatment effects such as the development of tolerance and treatment withdrawal reactions. Furthermore, now that additional functions of AChE have been identified, the effects of ChE-Is must be more broadly considered.

**Neuroanatomy of AChE and BuChE expression in the central nervous system**

**Neuronal locations**

The majority of ChE in the human brain is AChE. However, it is now known that BuChE has more
widespread distribution than previously thought (Mesulam et al., 2002a). In the normal brain, BuChE activity has been located in all regions that receive cholinergic innervation. It is mainly found in glial cells and in endothelial cells, whereas AChE is in neurons and axons (Mesulam et al., 2002a). Although AChE-containing neurons are widely distributed and more numerous than BuChE neurons, there is a particularly high expression of BuChE immunopositive neurons in the hippocampus, thalamus and amygdala (Darvesh et al., 1998, 2003a; Mesulam, 2000). In these structures, both AChE and BuChE have widespread but different distributions (Darvesh and Hopkins, 2003). For example, thalamic nuclei (anteroventral, mediodorsal, ventral anterior, lateral, and pulvinar) show particularly high BuChE expression; 90% or more neurons show intense immunostaining for BuChE (Darvesh and Hopkins, 2003).

Thalamic nuclei have both specific and non-specific projections to the cortex. The specific projections are usually from nuclei that show intense BuChE activity. As would be expected from the structures to which they project, pathological states of the subcortical nuclei in which BuChE-positive neurons are found have been implicated in working memory, attention, executive function and behaviour deficits (Darvesh and Hopkins, 2003). For example, the mediodorsal nucleus, which is rich in BuChE-positive neurons, projects mainly to prefrontal and cingulate cortices (Kievit and Kuypers, 1977), and this nucleus has a vital role in working memory, planning, and sequencing complex behaviours, all universal deficits in AD. In addition, the pulvinar sub-nuclei are involved in visual attention, a function that may be affected early in AD (Greenwood et al., 2000). Other thalamic nuclei that project to the frontal cortex and that, again, almost exclusively express BuChE include the anteroventral and lateral dorsal nuclei (Darvesh and Hopkins, 2003).

The role of glial cells

In the non-AD human brain, glial cells such as astrocytes, microglia and oligodendrocytes release AChE and BuChE into the extracellular space when activated. In patients with AD, intense BuChE and AChE activity is found in the vicinity of amyloid plaques, amyloid angiopathy, and neurofibrillary tangles (NFTs) (Mesulam et al., 1992; Wright et al., 1993), and these activities may stem from glia. In non-AD and AD brains, AChE-positive glia are widely distributed throughout the cortical layers and subcortical white matter, whereas BuChE-positive glia reach high densities only in the deep cortical layers and subcortical white matter (Wright et al., 1993). In the non-AD brain, the ratio of BuChE- to AChE-positive glia is higher in the entorhinal and inferotemporal cortex (two regions with a high susceptibility to AD pathology), than in the somatosensory and visual cortex (two regions with a relatively low susceptibility to the disease) (Wright et al., 1993). Gial BuChE/AChE ratios are increased in AD in the entorhinal and inferotemporal cortex, but not in other brain areas.

Gial cells produce a variety of trophic and neurotoxic factors, and regulate levels of synaptic neurotransmitters such as glutamate. In patients with AD both astroglia and microglia proliferate, particularly around plaques, and the profile of receptors that they express changes markedly (Meda et al., 2001). It is generally assumed that reactive gliosis is secondary to plaque formation, but a direct role for glia in plaque formation has also been proposed (Schubert et al., 2001). For example, Aβ activates glial cells to act as pro-inflammatory cells that secrete pro-inflammatory cytokines, which induce further Aβ deposition. Further studies to determine the relationship of BuChE and AChE with changes in receptor expression, cytokine release, and function in glial cells are needed to determine how BuChE and AChE may impact upon plaque maturation and disease progression. As gial BuChE may co-regulate ACh, elevated levels of BuChE in AD may exacerbate the reductions in cholinergic neurotransmission by further decreasing ACh levels.

Symptoms of cholinergic deficiency in dementia

A substantial cortical cholinergic denervation is a major aspect of advanced AD that is most severe in the temporal lobes and in adjacent limbic and paralimbic areas. Cholinergic innervation of the thalamus, originating in the brainstem, and cholinergic innervation of the striatum, originating from striatal interneurons, remain relatively intact. Therefore, the cholinergic lesion in AD is not generalized but selective. Moreover, recent studies indicate that loss of cholinergic markers are not detected in individuals with mild AD, and is a relatively late event during the course of AD (Davis et al., 1999; Minger et al., 2001; Rinne et al., 2003). Despite the absence of manifest cholinergic deficits, there is evidence that ChE-Is may be associated with symptomatic benefits in early AD (Peterson et al., 2005; Salloway et al., 2004).

Cholinergic deficits in the early stages of AD may relate to defects in cholinergic signal transduction (Haring et al., 1998; Mufson et al., 2000). Aβ may act as a physiological modulator of cholinergic function
(Auld et al., 1998) and low concentrations of soluble Aβ insufficient to induce neurotoxicity can inhibit ACh synthesis and release, resulting in cholinergic hypofunction, decreased neural efficiency and cognitive impairment. These effects seem to appear in the hippocampus and cortex but not in other brain areas (Kar et al., 1998). Basal forebrain cholinergic neurons, but not striatal or brainstem cholinergic neurons, are among the most sensitive cells in the brain to age-related neurofibrillary degeneration (Sassin et al., 2003). Thus, Aβ may be implicated in impaired basal forebrain cholinergic neurotransmission before levels of Aβ have achieved neurotoxic levels.

Ascending cholinergic pathways have an excitatory neuromodulatory effect on the cholinceptive neurons of the cerebral cortex, making them more susceptible to other incoming excitatory inputs (McCormick, 1990). Mesulam (2004) summarizes evidence that the neuroanatomy of cortical cholinergic innervation suggests crucial roles in the ‘modulation of attention (i.e. the on-line holding and dynamic enhancement of neural responses to salient events) and memory (i.e. the off-line encoding, retention, and retrieval of past events and contingencies)’. The former may reflect widespread cortical innervation and excitatory neuromodulation, while the latter may reflect a high level of cholinergic innervation of limbic areas, including the hippocampus, where the role of cholinergic innervation in modulating experience-dependent neuroplasticity may be of particular relevance (Mesulam, 1999). Although, cortical cholinergic pathways could potentially influence all aspects of cognition and behaviour, impaired cholinergic neurotransmission alone cannot fully account for the clinicopathological presentation of AD. The highly heterogeneous degeneration of multiple neurotransmitter pathways, along with multiple other mutually reinforcing neurodegenerative mechanisms, also underlies many of the symptoms of AD. It is, therefore, difficult to define the symptom profile of cholinergic deficit in dementia patients.

It has been proposed that cholinergic deficiency in the central nervous system is characterized by loss of attention/concentration and reduced capacity to detect and select relevant stimuli (Lemstra et al., 2003). These deficits can result in patients becoming restless, anxious and confused. Delusions and hallucinations may be a consequence of patients’ impaired perception of reality. Neurobiologically based models of human cognition distinguish between instrumental functions such as language, perception and praxis, and fundamental functions such as set shifting, attention/concentration and rate of information processing (Albert, 1978; Cummings, 1990). It has been suggested that effects on cholinergically mediated fundamental functions are responsible for the improvements in AD symptoms during treatment with ChE-Is (Lemstra et al., 2003). Instead of serving specific instrumental cortical functions, the diffusely organized system of cholinergic input to the cortex serves more fundamental roles of detection, selection, discriminating and processing of sensory stimuli and higher processes (Sarter and Bruno, 1997; Selden et al., 1998). A deficient cholinergic input system impairs efficient cortical processing and response to new and relevant stimuli. Fundamental factors such as motivation and attention/concentration modulate performance on instrumental neuropsychological tasks requiring perception, language and praxis. The fact that many patients with AD suffer mainly from impairments of instrumental functioning as a result of extensive cortical damage, and have relatively intact fundamental functioning, may explain the poor response to ChE inhibition seen in a significant proportion of AD patients. Assessments of selective and sustained attention and behaviour have rarely been conducted in randomized clinical trials in AD.

A diagnosis of AD or ‘dementia’ may be a poor descriptor of the target for ChE-I therapy. Rather than a nosological disease category, it may be better to identify ChE-I treatment with a ‘cholinergic deficiency syndrome’ of cholinergically mediated fundamental functions that are irrespective of the precise cause of neurodegeneration (Lemstra et al., 2003). The role of cholinergic projections from the basal forebrain in mediating cortical arousal via ACh release, enhancing cell responsiveness and increasing signal-to-noise ratio, shifts the cortical electroencephalogram (EEG) from the classical slow-wave pattern of high-amplitude, low-frequency oscillations to the desynchronized, higher-frequency, low-amplitude waveforms of wakefulness and arousal. In AD and related dementias, there is an increase in slow-wave (theta and delta) activity, a decrease in faster frequencies (alpha and beta), and a prolonged auditory P300 latency on the quantitative electroencephalogram (qEEG) – changes which are related to deficits in cholinergic neurotransmission (Adler et al., 2003; Braverman and Blum, 2003; Fogelson et al., 2003; Frodl-Bauch et al., 1999).

ChE-I treatment has been shown to enhance qEEG coherence with decreased slow-wave activity and increased faster frequencies reflecting increased cortical arousal, and providing improvements in concentration, sensory processing, and learning and memory (Adler and Brassen, 2001; Brassen and Adler, 2003; Fogelson et al., 2003). On an individual level, it has
Cholinergic neurotransmission

AD involves selective loss of cholinergic neurons in the brain. The inhibition of AChE and BuChE increases and maintains the synaptic concentration of ACh and allows greater interaction with receptors. In the presynaptic cholinergic neuron, ChAT catalyses the synthesis of ACh from choline and acetyl-coenzyme A. ACh is then packaged in synaptic vesicles for extracellular export. Action potentials arriving at the presynaptic nerve terminal trigger the release of ACh into the synaptic cleft, where ACh can interact with muscarinic and nicotinic receptors located on the pre- and post-synaptic membrane. Muscarinic M$_2$ receptors on the presynaptic membrane regulate ACh release via a negative feedback response. At the post-synaptic site, muscarinic M$_2$ receptors transduce signals through pathways in the post-synaptic neuron. ACh is hydrolysed in the synaptic cleft by membrane-bound tetrameric G4 AChE, or by soluble monomeric G1 AChE.

BuChE is also present in synapses and in the neuromuscular junction (Mesulam et al., 2002b), where it binds to the same structural synaptic membrane unit as AChE-S (Krejci et al., 1997). Thus, at the end of each episode of presynaptic secretion of ACh, binding of ACh to post-synaptic ACh receptors, and initiation of a post-synaptic action potential, BuChE may serve the same biological functions as AChE in the hydrolysis of ACh (Perrier et al., 2002). A high affinity choline-uptake mechanism returns choline to the pre-synaptic neuron. The close spatial relationship between glial cell protoplasm and the synaptic gap means that diffusing, extracellular ACh entering glial cells may be effectively hydrolysed by glial BuChE. This is analogous to the role of glia in glutaminergic neurotransmission, where they have a role in metabolizing excess glutamate.

The wide distribution of the central cholinergic pathways means that enhanced cholinergic neurotransmission can have broad effects on central behaviours and functions, including endocrine effects due to innervation of the hypothalamus. Both muscarinic and nicotinic receptors are present not only on cholinergic nerve terminals but also on monoaminergic nerve terminals. Systemic administration of ChE-Is in animal models has been demonstrated to significantly increase levels of norepinephrine, dopamine and serotonin (Cuadra et al., 1994; Giacobini et al., 1996; Mori et al., 1995; Warpman et al., 1996) that may be of clinical significance in ameliorating a variety of mood and anxiety symptoms in AD. Again, the effects on neurotransmitter levels may differ amongst the ChE-I group and in different brain regions (Trabace et al., 2001; Zhang et al., 2004). In addition to directly improving the cholinergically mediated symptoms of AD and indirect effects on multiple other neurotransmitters and neuroendocrine functions, enhanced cholinergic neurotransmission may mediate other important effects, including changes in CBF and amyloidogenesis (discussed later in this review).
The role of BuChE

Selective BuChE inhibition has been shown to elevate extracellular ACh levels and to improve learning in elderly rats performing maze trials (N. Greig, personal communication; Giacobini, 2004; Giacobini et al., 1996). Mice with no AChE activity (AChE knockout mice), are not only able to survive, but also exhibit structural integrity of central and peripheral cholinergic pathways, indicating that BuChE is capable of compensating for some functions of AChE (Mesulam et al., 2002b). Studies have also confirmed that BuChE is able to hydrolyse ACh in the non-AD human brain, and that it plays an important role in cholinergic neurotransmission (Mesulam et al. 2002a). Selective inhibitors of BuChE are not yet available for human testing. However, there is an increasing appreciation of the potential importance of BuChE in dementia (Giacobini, 2004; Greig et al., 2003).

The extent of BuChE inhibition in AD patients has been correlated with cognitive improvement (Darreh-Shori et al., 2002; Giacobini et al., 2002), particularly with improvements in verbal, spatial, memory, reaction time and speed tasks. The parallel influence of AChE and BuChE upon cholinergic neurotransmission explains why mice nullizygous for AChE and humans with enzymatically silent or greatly reduced BuChE can escape the potentially disastrous effects of cholinergic over-activity (Mesulam, 2003). Moreover, since AChE activity decreases and BuChE activity increases as AD progresses, the inhibition of BuChE may become an increasingly important therapeutic target over time (Arendt et al., 1992; Perry et al., 1978).

BuChE polymorphic variants

The presence of BuChE variants (K-variant homozygotes and A-variant heterozygotes) has been correlated with preserved attentional performance in a group of 58 patients with Lewy Body Dementia (LB) (McKeith et al., 2003; O’Brien et al., 2003; Figure 2). Moreover, these patients showed no significant improvements in attentional performance relative to placebo after 20 wk of treatment with the dual ChE inhibitor, rivastigmine. In contrast, rivastigmine treatment of individuals with one or two wild-type BuChE alleles showed significant improvements in attentional performance relative to placebo.

Particular polymorphisms of the gene for BuChE may alter the activity or the expression of the enzyme. Attentional performance in patients with BuChE variants was less impaired at baseline and demonstrated less response to rivastigmine, possibly due to these patients being close to the ceiling of their attentional performance at baseline, and having less BuChE activity to inhibit (Figure 2). K-variant BuChE has been proposed as a risk factor for AD (Lehmann et al., 2001), and the consequent dementia phenotype may have different symptom and ChE-I response characteristics compared with K-variant non-carriers with dementia. Evidence of deficits and treatment responses for symptoms other than attention in patients with dementia carrying BuChE polymorphisms is lacking, and care should be taken not to extrapolate this result to other cognitive and neuropsychiatric symptom domains. Reductions in attentional performance in individuals with BuChE variants were not seen in non-demented individuals with mild cognitive impairment (O’Brien et al., 2003), suggesting that the involvement of BuChE in attentional performance is limited to patients with dementia.

The basal forebrain cholinergic neurons project to all cortical areas, but only receive cortical inputs from the limbic system. Therefore, cortical cholinergic innervation may preferentially promote the allocation of attentional resources to events that are of emotional and motivational significance. Thus the potential involvement of BuChE in attentional processes correlates well with the neuroanatomical concentration of BuChE-positive neurons in the amygdala and hippocampus (Darvesh et al., 1998).

Figure 2. Attention deficit [single reaction time (SRT) in milliseconds (ms) ± standard error of the mean (S.E.)] in DLB patients by BuChE genotypes: ‘normal’ (wild-type) heterozygotes, K-variant heterozygotes, and a combined group of K-variant homozygotes and atypical heterozygotes. wt, Wild-type BuChE variant, normal enzyme activity; K, K-variant BuChE, reduced enzyme activity; A, atypical BuChE variant, reduced enzyme activity. (Adapted with permission from McKeith et al., 2003.)
ChE inhibition

Differences in ChE inhibition between agents

The profiles of ChE inhibition differ markedly amongst the ChE-Is (Table 1). In-vitro studies of potency and selectivity of ChE-Is are not a reliable indication of the situation in either the AD or non-AD brain (Poirier, 2002). Positron emission tomography (PET) ligand binding and CSF studies are required. The CSF bathes the brain and the spinal cord and, therefore, biochemical changes that occur in the brain are likely to be reflected in the CSF. Furthermore, AChE and BuChE activity in CSF primarily derives from brain (Atack et al., 1986; Chubb et al., 1976; Giacobini et al., 2002). A 6-wk CSF study in AD patients indicated approximately equivalent inhibition (~60%) of AChE and BuChE by 2–12 mg/d rivastigmine (Giacobini et al., 2002). In another study, after 12 months of 3–12 mg/d rivastigmine in AD patients, inhibition of CSF AChE and BuChE activity were 45% and 58% respectively (Darreh-Shori et al., 2002).

AChE PET ligand-binding studies of donepezil (5–10 mg/d) in AD patients have shown 27–39% inhibition after 4–6 wk (Kuhl et al., 2000; Shinotoh et al., 2001). An AChE PET ligand-binding study with 10 mg/d donepezil or 9 mg/d rivastigmine in AD patients demonstrated AChE inhibition of ~40% in the frontal cortex and ~30% in the temporal cortex for both drugs over 3–5 months (Kaasinen et al., 2002). This regional difference was possibly related to a reduced AChE activity at baseline in the temporal cortex. A BuChE PET ligand-binding study demonstrated BuChE inhibition of ~50% in the hippocampus and cortex with rivastigmine and negligible inhibition by 10 mg/d donepezil (Rinne et al., 2004). Galantamine (50 mg/d) for 3–6 months in healthy volunteers induced appreciable CSF inhibition of AChE but had no effect on BuChE activity (Thomsen and Kewitz, 1990).

Influence on safety and tolerability

In addition to alleviating the cognitive and behavioural deficits associated with deficits in cholinergic transmission, ChE inhibition may induce adverse effects. These may be acute symptoms associated with initiating therapy, or chronic symptoms associated with continued therapy. Although recent speculation on the clinical effects of BuChE inhibition has implicated it in acute adverse events such as nausea and vomiting (Wilkinson et al., 2002), data do not support this view (Darvesh et al., 2003b; Grossberg, 2003; Jhee et al., 2002). Rather, it is the rapidity and maximal increases in brain levels of ACh and brain-compensating mechanisms that increase dopamine, and not a specific BuChE inhibition, that are responsible for acute adverse events (Darvesh et al., 2003b; Grossberg, 2003).

For example, both physostigmine and metrifonate inhibit AChE and BuChE. Physostigmine, which is not well tolerated, inactivates both cholinesterases ~1000 times faster than metrifonate, the latter drug being well tolerated (Darvesh et al., 2003b). Peripheral dopamine blockade and peripheral anticholinergics do not influence tolerability. Only centrally acting dopamine-blocking anti-emetics appear to be effective at relieving ChE-I induced nausea and vomiting (Jhee et al., 2002).

Adverse effects, other than centrally mediated nausea and vomiting, that may lead to treatment discontinuation of ChE-Is during longer term therapy may be associated with a lack of central vs. peripheral selectivity (e.g. muscle cramps) or brain regional selectivity (e.g. sleep disturbances, extrapyramidal symptoms), due to a lack of preferential activity on disease-relevant ChE molecular forms. The distribution of AChE isoforms may vary between brain regions and in the periphery. For example, relative to other brain regions there are greater amounts of the G1 molecular form of AChE located in the cortex, amygdala and hippocampus, and G1 AChE is also associated with neuritic plaques. The G4 molecular form of AChE is the predominant form in the neuromuscular endplate, and is co-localized with a variable proportion of G2 AChE.

Donepezil may be associated with a relatively high incidence of insomnia, vivid dreams, hypnotic use, muscle cramps, rhinitis, and urinary incontinence (Grossberg, 2003; Salloway et al., 2004; Stahl et al., 2003; Wilkinson et al., 2003). In addition, although relatively rare in clinical practice bradycardia may develop with donepezil (Bordier et al., 2003; Calvo-Romero and Ramos-Salado, 1999), that in overdose this may be protracted (Shepherd et al., 1999), and donepezil may adversely influence cardiovascular autonomic control with reductions in heart rate variability (McLaren et al., 2003; Masuda and Kawamura, 2003). Rivastigmine is preferentially selective for the G1 form of AChE and does not induce overall increases in AChE activity (Darreh-Shori et al., 2002; Enz, 1993; Rakonczay, 2003). Galantamine does not appear to target particular molecular forms of AChE (Rakonczay, 2003), but its competitive inhibition of AChE may confer a reduced potential for inhibition of neuromuscular or brainstem AChE in the absence of ACh deficiency.
Hypothalamic cholinergic neurotransmission plays a major role in the regulation of growth hormone (GH) secretion, and blockade of muscarinic receptors blunts the release of GH (Diez et al., 2000). The secretion of GH and insulin-like growth factor 1 (IGF-1) decline by 14% with each decade of adult life. Senile GH deficiency, termed somatopause, is manifested by loss of muscle, bone mass and cardiac function, and an increased prevalence of atherosclerosis (Rehman and Masson, 2001). Acute administration of ChE-Is can decrease hypothalamic somatostatinergic tone, release GH, and enhance the GH response to GH-releasing hormone (GHRH) (Obermayr et al., 2003). GH and GHRH may improve memory function (Deijen et al., 1998; Schneider-Rivas et al., 1995), and IGF-1 may improve perceptual motor and processing speed (Aleman et al., 1999). It has, therefore, been suggested that some of the cognitive effects of ChE-Is may be mediated by increased concentrations of GH or IGF-1 (Obermayr et al., 2003). Future investigations are necessary to find out whether ChE-Is can restore the senile decline of circadian GH secretion in the long term, and to assess potential new implications for patient treatment (Obermayr et al., 2003).

**Disease progression: possible mechanisms of ChE-Is**

It is important to emphasize the difference between ‘slowing’ and ‘delaying’ disease progression. All ChE-Is have demonstrated short-term symptomatic effects which provide actively treated patients with a higher baseline from which they subsequently decline. This effect results in transiently delaying patient decline. However, ideally, agents should also slow decline – demonstrated by a widening gap over time between the courses of treated and untreated patients. It is the latter effect only that provides evidence of disease modification. Such effects have only been suggested in post-hoc analyses (Doraiswamy et al., 2002; Farlow et al., 2000, 2003), and there is, as yet, no definitive evidence that any of the ChE-Is modify the underlying disease process.

There are a number of mechanisms whereby ChE-I might potentially produce disease-modifying effects. It has been suggested that activation of nerve cells leads to maintenance of neurons during ageing and in AD. This ‘use it or lose it’ principle suggests that neuronal activation might provide a means of prolonging optimal neuronal function, the survival of remaining neurons, and modification of the course of the disease. Classical cholinergic signal transduction pathways may protect against neuronal degeneration by modifying the formation of amyloidogenic compounds and decreasing tau phosphorylation (Fisher et al., 2003; Hellstrom-Lindahl, 2000), reducing neuronal vulnerability to Aβ toxicity (Kihara et al., 2001), promoting neurotrophin release (Isacson et al., 2002), and reversing the changes in rCBF and glucose metabolism that occur very early in the course of the disease (Kirkpatrick et al., 2003; Stefanova et al., 2003). Non-classical effects of AChE and BuChE include the induction of Aβ fibrilization (Inestrosa et al., 1996), plaque maturation (Darvesh et al., 2003b; Geula and Mesulam, 1995), activation of the cytokine cascade (Reale et al., 2004), and increased neurite outgrowth (Whyte and Greenfield, 2003). In addition, preliminary evidence indicates that ChE-Is may modulate gluatamate and other biogenic amine neurotransmission in the brain, and this could represent an additional mechanism of action in AD (Trabace et al., 2001; Zhang et al., 2004). It is not known which, if any, of these mechanisms are most likely to contribute to a clinically meaningful disease-modifying effect or whether these effects would be seen with dosages of cholinesterase inhibitors used in clinical practice.

**Cholinergic neurotransmission and the amyloid cascade and tau phosphorylation**

Cholinergic neurotransmission, and both AChE and BuChE, may have important interactions with the amyloid cascade. For example, the enhancement of cholinergic mechanisms favours non-amyloidogenic processing of amyloid precursor protein (APP) (Beach et al., 2001; Fisher et al., 2002; Greig et al., 2001; Guillozet et al., 1997; Soreq and Seidman, 2001). In-vitro and in-vivo studies have consistently demonstrated a link between cholinergic activation and APP metabolism (Giacobini, 2003). For instance, experimentally or pathologically (e.g. in AD) reduced cholinergic neurotransmission leads to amyloidogenic metabolism (Wallace et al., 1991, 1995). Furthermore, Aβ neurotoxicity is attenuated by muscarinic agonists (Emmerling et al., 1997). Chronic administration of anti-muscarinic drugs may accelerate the course of AD (Lu and Tune, 2003), and increase the burden of AD-type pathology, such as Aβ deposits and NFTs (Perry et al., 2003a). Lesions of cholinergic nuclei cause a rapid increase in cortical and CSF levels of APP and this effect can be reversed by ChE-I treatment (Haroutunian et al., 1997). Muscarinic agonists such as ACh, which stimulate the M4 receptor promote non-amyloidogenic APP-processing pathways and decrease tau protein...
phosphorylation (Fisher et al., 2000; Nitsch et al., 1998). Hyperphosphorylation of tau microtubule-associated protein may represent a link between the cholinergic signal transduction systems, disruption of the neuronal cytoskeleton in AD, and the genesis of NFTs (Sadot et al., 1996). M1 and M4 mAChR-mediated signal transduction activate protein kinase C (PKC), and the mitogen-activated protein kinase (MAPK)-dependent pathways, reducing Aβ production by stimulating α-secretase cleavage of APP and the secretion of non-amyloidogenic soluble APP (sAPPα) (Beach et al., 2001; Fisher et al., 2003; Haring et al., 1998), which is neuroprotective and neurotrophic (Hooper and Turner, 2002). In the alternative, amyloidogenic pathway, β-secretase cleaves APP releasing sAPPβ and a C-terminal fragment (CTFβ), which can be further cleaved by γ-secretase to form Aβ, which is released into the extracellular space. A decrease in α-secretase and a large increase in β-secretase activity have been reported in the temporal cortex in patients with AD (Tyler et al., 2002). PKC may also inhibit glycogen synthase kinase-3 (GSK-3β) (Forlenza et al., 2000). This may decrease tau phosphorylation, further decrease amyloidogenesis, and decrease the neurotoxic effects and apoptosis induced by Aβ (Planel et al., 2002).

Stimulation of nicotinic cholinergic receptors (nAChRs) may also influence APP processing towards reduced production of amyloidogenic sAPPγ (Utsuki et al., 2002). Furthermore, although muscarinic receptor density is little changed in AD, functional studies have shown impairment of M1 AChR receptor-mediated signal transduction. The M1 AChR is the major post-synaptic receptor of the cerebral cortex. Low concentrations of Aβ may disrupt signal transduction through M1 mAChR receptors (Kelly et al., 1996), and this uncoupling may lead to a decrease in sAPPα secretion, generation of more Aβ, and a ‘vicious cycle’ of further cholinergic deficiency (Fisher et al., 2002).

Evidence for ChE-I-induced increases in cholinergic neurotransmission affecting tau and amyloid pathology in AD patients is limited. However, in a recent study, no change was seen in CSF tau levels after 1 yr in rivastigmine-treated AD patients, while significant increases were seen in untreated AD patients (Stefanova et al., 2003). If clinical correlates were to be demonstrated, the involvement of cholinergic receptor-mediated signal transduction pathways in amyloidogenesis and tau phosphorylation might prompt revision of the cholinergic hypothesis in AD, which currently ignores the potential of cholinergic therapies as disease-modifying agents.

**Allosteric nAChR modulation**

The ChE-I, galantamine, has been shown in vitro to act as an allosterically potentiating ligand on particular subtypes of the human nAChR, such as the α4/β2 subtype (Samochcki et al., 2000). The clinical relevance of this finding is unclear. Loss of nAChRs occurs early in AD, particularly in patients with APOE ε4 alleles. The established efficacy of galantamine in subgroups of patients with moderately severe AD, and in AD patients with APOE ε4 alleles is probably due to AChE inhibition alone (Aerssens et al., 2001; Blesa et al., 2003). All ChE-Is indirectly interact with nAChRs through the elevation of ACh.

The marked loss of nAChRs that occurs in the AD brain (Kellar et al., 1987) has been shown to be prevented over the course of 1 yr or more in patients with mild AD treated with tacrine, which has no apparent ability to allosterically modulate nAChRs (Nordberg et al., 1998). AD patients showed increased nicotinic receptor binding in the temporal cortex, as measured by PET, after 3 months of treatment with rivastigmine and to a lesser extent also with tacrine (Nordberg, 2004). The lesser effect of tacrine may relate to its decreased ability to behave as an open channel blocker of nicotinic receptors (Prince et al., 2002). Recent data have also shown that donepezil may block or desensitise nAChRs through a direct interaction of donepezil with nicotinic receptors (Di Angelantonio et al., 2004).

**Amino acidergic transmission**

There has been considerable interest in excitatory amino-acid theories of neurodegenerative disease such as AD (Nakanishi et al., 1998). Glutamate is the major excitatory neurotransmitter in the CNS and can induce excitotoxicity. In fact, in AD, cortical and hippocampal pyramidal neurons, which are particularly affected by NFTs and neuronal degeneration, use glutamate or aspartate as neurotransmitters (Maragos et al., 1987). Moreover, it has been shown that the distribution of senile plaques in the AD brain correlates with the distribution of glutaminergic synapses (Rogers and Morrison, 1985). Glutamate signal transduction at the post-synaptic terminals is initiated by stimulation of glutamate receptors, especially of the N-methyl-D-aspartate (NMDA) subtype. Aβ can enhance the release of glutamate, prevent its uptake by synaptosomes and glia, and increase neuronal and glial cell sensitivity to glutamate-induced excitotoxicity. A degenerative feedback cycle may be generated of excess glutamate, induction of NMDA receptor-mediated depolarization and intracellular
Ca\(^{2+}\) increases, with further glutamate release, and loss of neuronal function. Excessive intracellular Ca\(^{2+}\) accumulation leads to an abnormal activation of Ca\(^{2+}\)-dependent enzymes, such as neuronal nitric oxide synthase (nNOS). This enzyme catalyses the formation of nitric oxide (NO) and citrulline from L-arginine. Taken together, these data suggest a role for amino acids in the clinical manifestation and pathogenesis of AD (Nakanishi et al., 1998) as an alternative, not necessarily exclusive, to the cholinergic hypothesis.

The effects of oral rivastigmine, an inhibitor of AChE and BuChE, and CHF2819, a selective inhibitor of AChE, on extracellular concentrations of amino acids in the rat hippocampus, were evaluated using in-vivo microdialysis (Trabace et al., 2001). Rivastigmine, but not CHF2819, significantly decreased glutamate, arginine and citrulline levels, without affecting aspartate concentrations. In addition, 3- and 21-d treatment with rivastigmine increased the expression of the neuronal glutamate transporter in the rat hippocampus but not in cortical areas (Andin et al., 2004). These results suggest that the modulation of the glutaminergic system could represent an additional mechanism of action in AD for ChE-Is.

\(\beta\) fibrilization

It has been observed that in AD, brain cortical AChE activity is associated predominantly with the amyloid core of mature senile plaques (Alvarez et al., 1998). Therefore, AChE might have a crucial role in amyloid deposition at an early stage of the disease. One of the central events in the AD pathogenesis is the \(\beta\) fibrilization, and both BuChE and AChE may have roles in the aggregation of \(\beta\). AChE may influence the in-vitro and in-vivo aggregation state of \(\beta\), perhaps through the non-catalytic mechanisms related to the PAS subsite (Bartolini et al., 2003; De Ferrari et al., 2001; Inestrosa et al., 1996; Lahiri et al., 2000). AChE may constitute an important co-factor in \(\beta\) fibrillogensis, being able to induce a conformational change of \(\beta\) in solution and thus, having a direct role in fibril formation. Moreover, AChE-\(\beta\) complexes have been shown to be more neurotoxic than \(\beta\) peptides alone (Alvarez et al., 1998; Opazo and Inestrosa, 1998), and may be involved in the pathogenesis of AD (Opazo and Inestrosa, 1998). Thus, both in-vitro and in-vivo data suggest that AChE behaves as a potent amyloid-promoting factor and modulates the toxicity of amyloid fibrils. AChE-induced aggregation was found to be inhibited by peripheral anionic site ligands such as propidium, and only partially inhibited by molecules binding both to the catalytic site and to the peripheral site such as physostigmine and donepezil (Bartolini et al., 2003; Piazz et al., 2003).

Pathological amino-or N-terminal-truncated species of \(\beta\)42 may be involved in the earliest stages of amyloidogenesis in humans (Sergeant et al., 2003). N-terminal-truncated pyroglutamyl-ended forms of \(\beta\) are present in senile plaques and vascular walls in individuals with AD (Kuo et al., 1997; Saido et al., 1995). Pyroglutamyl-ended \(\beta\) peptides (\(\beta\) pE) form \(\beta\)-sheet structures more readily than the corresponding full-length \(\beta\) peptides (He and Barrow, 1999). Interestingly, experimental data suggest that ChE-Is may avoid the formation of \(\beta\) pE deposition through the activation of pyrrolidone carboxyl peptidase (Pcp). The activity of this enzyme is reduced in the plasma of sporadic AD (Kuda et al., 1997). Acute administration of rivastigmine increased Pcp activity in frontal cortex synaptosomes from mice in a dose-dependent manner (Ramirez-Exposito et al., 2001).

\(\beta\) fibrilization

The factors that contribute to the transformation of a relatively inert diffuse plaque to a neuritic or pathogenic one may involve interactions with additional plaque constituents (Guillozet et al., 1997). Such constituents include the G1 isoforms of both AChE and BuChE (Geula and Mesulam, 1995). The diffuse amyloid plaques of normal ageing tend not to display BuChE activity, whereas the vast majority of compact neuritic plaques do (Geula and Mesulam, 1995). The AChE and BuChE in these pathological structures has altered sensitivity to ChE-Is and to proteolytic enzymes (Geula and Mesulam, 1995; Wright et al., 1993). BuChE and AChE may interact with other proteins to form complexes that could serve a regulatory role (Darvesh et al., 2001, 2003b). It has been suggested that AChE might have a crucial role in amyloid deposition at an early stage of the disease (Alvarez et al., 1998), and based upon these observations, it seems that BuChE may play a role in the transformation of benign plaques to a malignant form associated with neuritic tissue degeneration and clinical dementia. Furthermore, the substantial increase in BuChE activity in more pathogenic forms of \(\beta\) deposits raises the possibility that the in-vivo imaging of BuChE may have potential as a diagnostic tool.

There is an emerging concept that the net biological response of pro- and anti-inflammatory cytokines affects the outcome of certain diseases including neurodegenerative disorders (O’Shea et al., 2002). Transcription and production of pro-inflammatory cytokines are markedly enhanced in AD (Reale et al.,
and protective in female non-APOE e with APOE (Alvarez-Arcaya et al., 2000). BuChE-K may interact males with an APOE e suggestion that BuChE-K is a risk factor for AD in progression than non-carriers is not incompatible with the findings in patients with AD or DLB, show- ing that the presence of one or more BuChE-K alleles was associated with a significantly less rapid rate of cognitive decline over a 2- to 3-yr period than that found in individuals who were wild type (wt) for the BuChE gene. Cognitive decline was $9.6\pm 11.6$ CAMCOG points in patients with a K/wt or K/K phenotype, compared with $16.5\pm 11.8$ points in wt/wt patients ($p=0.04$). In a linear regression analysis evaluating the impact of diagnosis (DLB vs. AD), the BuChE genotype remained independently and statistically significantly associated with the rate of cognitive decline. A further study in patients with AD has confirmed these findings (Holmes et al., In Press).

These data support the hypothesis that BuChE may be an important moderator of AD progression (Ballard and Perry, 2003). The finding that carriers of a BuChE-K allele have differential rates of disease progression than non-carriers is not incompatible with the suggestion that BuChE-K is a risk factor for AD in males with an APOE e4 allele (Lehmann et al., 2001), and protective in female non-APOE e4 carriers (Alvarez-Arcaya et al., 2000). BuChE-K may interact with APOE e4 to increase the risk of dementia, but once AD is established carriers of a BuChE-K allele generally show less rapid progression of dementia compared to non-carriers (Holmes et al., In Press; O’Brien et al., 2003). Thus, evidence suggests that BuChE activity may have a role in disease progression in AD.

**Clinical evidence for a role of BuChE in disease progression**

The increases in BuChE in key brain areas may also provide a possible explanation for the accelerated rates of atrophy seen in patients with more severe AD. Perry and colleagues (2003b) reported a significant correlation between grey-matter BuChE levels in the temporal cortex and annual cognitive decline on the MMSE in a prospectively studied autopsy-confirmed DLB case series. If BuChE variants can affect attentional performance, possibly through the effect of BuChE on levels of available ACh, it is possible that BuChE variants may also have an effect upon disease progression as measured by the rate of cognitive decline.

O’Brien and colleagues (2003) recently presented data from a study of patients with AD or DLB, showing that the presence of one or more BuChE-K alleles was associated with a significantly less rapid rate of cognitive decline over a 2- to 3-yr period than that found in individuals who were wild type (wt) for the BuChE gene. Cognitive decline was $9.6\pm 11.6$ CAMCOG points in patients with a K/wt or K/K phenotype, compared with $16.5\pm 11.8$ points in wt/wt patients ($p=0.04$). In a linear regression analysis evaluating the impact of diagnosis (DLB vs. AD), the BuChE genotype remained independently and statistically significantly associated with the rate of cognitive decline. A further study in patients with AD has confirmed these findings (Holmes et al., In Press).

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**The influence of ChEs on cerebrovascular responses**

**Cholinergic neurotransmission and rCBF**

The integrity of the cerebral vasculature is crucial for the maintenance of cognitive function. Cerebrovascular function declines with age due to microvascular pathology, including amyloid deposition (Kalaria and Ballard, 1999) and are more evident in AD. In addition, cortical microvessels and NO-synthesizing interneurons in the AD brain exhibit severe cholinergic denervation (Tong and Hamel, 1999). Cholinergic neurons may be particularly susceptible to hypoxia, and chronic vascular hypoperfusion has been implicated in the pathogenesis and accelerated course of AD (Aliev et al., 2003).

Cholinergic neurons projecting from both parasympathetic ganglia and basal forebrain innervate cerebral blood vessels (Toda and Okamura, 2003). Inhibition of ChE increases rCBF through the dilation of intracortical microvessels (Kasa et al., 2000), and ablation of basal forebrain cholinergic neurons reduces rCBF and increases cortical vascular Aβ deposition (Roher et al., 2000). The cerebral vasodilatory effects of cholinergic activation may be mediated through cholinergic nerve terminals in contact with glial cells surrounding microvessels (Chedotal et al., 1994). In addition, many cholinergic neurons synthesize and release the potent vasodilator NO, and nitrergic neurons may receive inputs from cholinergic neurons (Toda and Okamura, 2003). It has been suggested that the cholinergic system plays an important role in the coupling mechanism between neuronal activity and functional responses of rCBF (Ogawa et al., 1994), and may also have a role in ensuring that the cortex and hippocampus have a sufficient blood flow during exercise (Kimura et al., 1994; Nakajima et al., 2003). Thus, cholinergic and nitrergic innervation, and the interaction between these systems, play a major role in the regulation of cerebrovascular tone (Toda and Okamura, 2003).

Recent evidence suggests that abnormalities in rCBF and energy metabolism are early changes in AD and may precede plaques, NFTs, and the appearance of neurodegeneration in AD (Gibson, 2002; Salmon et al., 1994). For example, decreases in brain metabolism are seen many years before the development of symptoms in patients predisposed to AD (Small et al., 2000), and this may reflect a very early dysfunction in
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Conclusion

The classical role of ChE is in the termination of ACh-mediated neurotransmission. Cholinergic neurotransmission is reduced in AD and it may be enhanced by ChE-I-induced increases in the synaptic availability of ACh. AChE inhibition may result in general improvements of cortical instrumental functions in patients with AD, as a result of improved underlying fundamental functions mediated by the widespread input of the AChE-positive neurons to the cortex. BuChE inhibition may result in additional specific input into frontal cortical structures due to the more focused projections of BuChE-positive thalamic neurons to frontal brain areas and the predominant neuroanatomical localization of BuChE in the limbic system and subcortical regions. The clinical expression of the differences between AChE-specific and dual ChE inhibitors will only be fully elucidated in well-designed comparative studies that assess improvements in underlying fundamental functions with sensitive instruments, and in patient populations with significant fundamental functional deficits. Determination of the effects of BuChE-specific inhibition awaits the assessment of BuChE-specific inhibitors in AD patients.

Relative potencies and ratios of AChE/BuChE inhibitory activity are not the only determinants of individual ChE-I clinical profiles. Pharmacokinetic characteristics, mechanisms of enzyme inhibition, brain selectivity, and differential selectivity for various molecular forms of each ChE may also be important factors. There are marked differences amongst ChE-Is in their potential to up-regulate AChE gene expression and in their modulation of alternative splicing of the AChE gene to produce changes in the composition of AChE molecular forms. Furthermore, as the severity of dementia advances, BuChE activity progressively increases while AChE activity declines. Long-term clinical studies, ideally comparing agents that inhibit BuChE alone, both BuChE and AChE, and AChE alone are needed to ascertain whether these differences amongst ChE-Is are reflected in differences in symptom control at various disease stages.

Further investigation is required to clarify the potential mechanisms whereby the non-classical roles of BuChE and AChE may affect disease progression in dementia. Many recent studies concern the regulation of APP processing and modulation of tau phosphorylation by ACh receptor stimulation, and how cholinergic deficits and Aβ production may be related to one another. In addition, AChE may have important roles in amplifying Aβ toxicity and in Aβ fibrilization.
Specific expression of AChE and BuChE in neurons, glia and endothelial cells suggests that, in addition to co-regulation of cholinergic neurotransmission, BuChE and AChE may also influence cholinergically and non-cholinergically mediated cerebral vascular responses. Normalization of the disrupted coupling mechanism between neuronal activity and rCBF observed in AD could delay the progressive decline in cognitive function, and might possibly reduce the formation of Aβ.

Neuromaging studies, transgenic animal models and autopsy studies of amyloid burden are required to determine the differential effects of BuChE and AChE inhibition on the neuronal and non-neuronal cholinergic systems. The physiological roles of the different splice variants or molecular forms of BuChE and AChE also require further clarification. Screening for protein partners that interact with various molecular forms of AChE and BuChE may identify possible biochemical pathways in which they participate. These promising leads on the non-classical and non-synaptic roles of AChE and BuChE in the modulation of AD and related dementias clearly require further investigation and offer the potential for improved treatments.

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Statement of Interest

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