Expert Opinion

Phenserine

Jochen Klein
University of Frankfurt College of Pharmacy, Biocenter N260, Max-von-Laue-Str. 9, D-60438 Frankfurt, Germany

Phenserine, a derivative of physostigmine, was first described as an inhibitor of acetylcholinesterase (AChE) and was shown to improve cognition in various experimental paradigms in rodents and dogs. It was clinically tested for Alzheimer's disease, with moderate success in initial Phase II studies. Phenserine deserves attention for an additional quality of action: in addition to inhibiting AChE, it modulates the amount of β-amyloid precursor protein (APP) in neuronal cell culture by reducing APP translation. This effect probably involves interaction of phenserine with a regulatory element in the 5′-untranslated region of the *APP* gene that controls APP expression. Phenserine apparently reduces translational efficiency of APP mRNA into protein, a process that may involve an interaction with iron and/or an iron-responsive element. As a consequence, phenserine reduces β-amyloid peptide (Aβ) formation *in vitro* and *in vivo*. Phenserine is also unique because of differing actions of its enantiomers: (-)-phenserine is the active enantiomer for inhibition of AChE, whereas (+)-phenserine ('posiphen') has weak activity as an AChE inhibitor and can be dosed much higher. Both enantiomers are equipotent in downregulating APP expression. (+)-Posiphen may be a promising drug, either alone or in combination with (-)-phenserine, to attenuate the progression of Alzheimer's disease.

Keywords: acetylcholine, acetylcholinesterase, Alzheimer's disease, amyloid peptides, amyloid precursor protein, cholinergic dysfunction, cholinesterase inhibitor, dementia, phenserine, posiphen

Expert Opin. Investig. Drugs (2007) ***16****(7):1087-1097*

1. Introduction

1.1 Alzheimer's disease: etiology and therapeutic targets

Classical signs of Alzheimer's disease, as described by Alois Alzheimer himself in 1906, are brain atrophy and neuronal cell loss, deposition of amyloid plaques in the brain extracellular space, formation of intracellular neurofibrillary tangles and, of course, the clinical symptoms of dementia (i.e., loss of attention, memory, and intellectual and cognitive processes). Dementia correlates with neuronal cell death in the Alzheimer's disease brain but the relationship between neuropathology and neurotoxicity is poorly understood (see Section 1.5). Systematic investigations of the amyloid plaques started in the 1980s and led to the identification of fibrillar, insoluble peptides, the so-called β-amyloid peptides ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$), which are generated by proteolytic cleavage from amyloid precursor protein (APP) and which form the core of amyloid plaques. Work in the last 20 years has also provided strong evidence that amyloid formation in the brain is a causal factor for disease etiology [1]. Rare familial cases of Alzheimer's disease have been identified that carry genetic mutations of *APP* or *APP* gene duplications; these mutations cause enhanced formation of amyloid peptides. More frequent among familial Alzheimer's disease cases are mutations in presenilin-1 and -2; presenilin-1 has been identified as part of the γ-secretase complex that, together with β-secretase, is responsible for the pathologic processing of APP to amyloid peptides. In contrast, processing of APP by α-secretase prevents amyloid peptide formation.

informa
healthcare

1.2 Amyloid hypothesis of Alzheimer's disease: some pros and cons

Several arguments have been put forward to support or dismiss the 'amyloid hypothesis of Alzheimer's disease' [2,3]. For example, APP is located on chromosome 21; trisomy 21 causes Down's syndrome and is associated with Alzheimer's disease-like pathology. Plaque densities in Alzheimer's disease brains were found to correlate poorly with clinical symptoms ([2] but readers are referred to [4]) and plaques also occur in cognitively normal elderly patients. Plaques may not be the responsible species for neurotoxicity; however, amyloid peptides were found to be the neurotoxic species in neuronal cell cultures and present data indicate a leading role for $A\beta_{1\text{-}42}$ or one of its aggregation states as the major culprit [3]. The physical forms of amyloid peptides in brain tissue are difficult to investigate, but present thinking favors a toxic role of Aβ peptide oligomers [5]. Several pathways have been suggested to explain how amyloid peptides affect neurons, including pro-apoptotic effects and mechanisms targeting synaptic transmission [6]. A 'modified β-amyloid hypothesis' focuses on the toxicity of amyloid peptides that are formed intracellularly [7]. A problem of many early experimental studies is the use of high (micromolar) concentrations of amyloid peptides, which may not occur *in vivo* [2].

1.3 Mouse models of Alzheimer's disease

Animal models of Alzheimer's disease were created by incorporating human genes, with or without familial Alzheimer's disease mutations, into the germline of mice. The models gave mixed results with respect to the amyloid hypothesis [8]. On the one hand, overexpression of mutated APP caused amyloid plaque formation, in particular when it was combined with expression of mutated presenilin-1 (although this combination does not usually occur in familial Alzheimer's disease). On the other hand, neurofibrillary tangles were not observed in these double transgenic mice, except when a mutated gene of the τ protein (which is hyperphosphorylated in Alzheimer's disease) was introduced to create triple transgenics [9]; again, it should be noted that parallel mutations of these three genes do not occur in human disease, which questions the validity of this approach. More importantly, it was difficult to identify neurotoxicity in many of these mouse models. Mice transgenic for APP often did not show neuronal loss, whereas others showed minor deficiencies at a later age (see Section 1.5 for cholinergic markers). Cognition was impaired in some mouse models, suggesting impairment of synaptic transmission, but cognitive testing in mice is known to be difficult and fraught with artifacts. Better evidence was provided by changes of long-term potentiation, an electrophysiological surrogate for memory formation, which (in the hippocampus) depends on glutamatergic or cholinergic input [10].

Final proof of the amyloid concept awaits the experimental and clinical testing of specific inhibitors of β- and γ-secretase, the two enzymes that are responsible for amyloid peptide formation. Strong proof of the amyloid hypothesis would be furnished if these inhibitors reduced amyloid load and improved cognition at the same time. However, the future clinical use of these inhibitors has been put to doubt because of potential adverse side effects [11].

1.4 Alzheimer's disease: the cholinergic link

Several types of neurons show degeneration in advanced Alzheimer's disease, including glutamatergic and 5-HT-mediated neurons. However, an extensive literature supports damage to the central cholinergic systems as having the best correlation with clinical dementia [12]. While some cholinergic fibers (e.g., striatal interneurons) remain healthy, severe degeneration is observed for the long cholinergic projection neurons that originate in the basal forebrain and innervate the cortex and hippocampus. Indeed, the cholinergic fibers that deteriorate in Alzheimer's disease are known to be required for processes of attention, learning and memory (i.e., those higher cognitive functions that are lost early in dementia). Those cholinergic neurons that originate in the *Nucleus basalis Meynert* and innervate the cortex evidently control attentional states and link motional and motivational pathways with cortical activation [13]. Septohippocampal cholinergic fibers can induce a specific θ rhythm and a cholinergic type of long-term potentiation in the hippocampus, and are required for formation of new memories [14].

Damage to the cholinergic system in experimental animals, as well as treatment with muscarinic antagonists, have been shown to induce cognitive deficits that could be alleviated by the administration of cholinesterase inhibitors (ChEIs) [15]. ChEIs were also effective to treat behavioral deficits in mouse models of Alzheimer's disease [16]. There is a link to growth factors for cholinergic systems: cholinergic dysfunction and dementia could be induced by a deficiency of nerve growth factor (NGF), a required growth factor for cholinergic projection neurons in the brain [17]. NGF-secreting cells are being developed for therapeutic purposes [18]. Moreover, a link between APP overexpression and inhibition of NGF axonal transport has been suggested [19]; this finding may explain the selective vulnerability of cholinergic neurons in Down's syndrome and, possibly, Alzheimer's disease.

1.5 Effects of amyloid on cholinergic function

The link between Alzheimer's disease neuropathology (such as amyloid plaques and neuro-fibrillary tangles) and cholinergic cell loss was difficult to establish. In favor of a causal connection, amyloid peptides were found to interfere with the synthesis and release of acetylcholine (ACh), as well as with choline homeostasis and postsynaptic muscarinic and nicotinic signaling in neuronal cell cultures and brain slices [20,21]. Injection of amyloid peptides into the brain caused a moderate reduction of ACh release *in vivo* but high concentrations of amyloid peptides were often used in these studies [22,23]. Mice that serve as models of amyloid formation showed reductions of cholinergic markers in some studies but not in others [24,25]. Microdialysis studies that reflect cholinergic activity *in vivo* reported both normal and reduced levels of ACh;

the reduced level was found in animals with a very high load of amyloid plaques [26]. In one study [27], reductions of ACh levels could be restored by treatment with antiamyloid antibodies, indicating that soluble amyloid peptides may interfere with cholinergic function. These findings are in agreement with reports of rapid amyloid peptide dynamics in the brain [28]. It should be noted that microdialysis studies (such as [25-27]) only reflect presynaptic cholinergic function; an impairment of postsynaptic cholinergic signaling, as recently described in Alzheimer's disease [29], would not have been detected.

Overall, in experimental studies, cholinergic deficits were more likely in older mice with high amyloid peptide levels. However, recent anatomical and histochemical studies in transgenic mice suggested that cholinergic damage may occur early in the disease as reductions of synaptic boutons, dendritic spine density and aberrant sprouting were found to occur in cholinergic neurons before plaque deposition could be seen [30,31]. These early pathological changes may be masked in studies of ACh release because the cholinergic system has a high capacity to compensate ACh release under various conditions (e.g., after changes of AChE activity) [32,33]. Clinical studies support the findings of cholinergic compensation in early Alzheimer's disease; severe cholinergic deficits become more visible in later stages of the disease [34].

1.6 Effects of cholinergic function on amyloid

A separate consideration is whether cholinergic treatment, once initiated, can affect the formation or toxicity of amyloid peptides. It is well known that cholinergic stimulation via muscarinic receptors can cause activation of α-secretase and, therefore, enhance the non-amyloidogenic pathway of APP processing [35]. Additional work demonstrated that the same effect can be observed with ChEIs [36]. Later work showed that this effect is not specific to the cholinergic system; stimulation of other receptors that are coupled to the phospholipase C second messenger pathway, as well as electrical stimulation of brain slices and even direct stimulation of protein kinase C with phorbol esters, activate α-secretase [37]. In contrast, activation of receptors that stimulate cyclic AMP (cAMP) production increases γ-secretase activity [38].

1.7 Additional sites for interaction of cholinergic drugs with Alzheimer's pathology

AChE occurs as one gene but in several splice variants; the transcription of individual splice variants is influenced by cellular stress and by ChEIs such as rivastigmine [39]. AChE is known to interact with amyloid peptides and is a prominent constituent of amyloid plaques. AChE has a peripheral site that binds amyloid peptides and favors their aggregation [40]. This has been shown *in vitro* as well as *in vivo* in mice that were transgenic for APP and human AChE [41]. Theoretically, ChEIs that interact with the peripheral site could delay amyloid deposition; however, the ChEIs in present therapeutic use (see Section 2) do not seem to efficiently interact with this binding site [42]. Dual-action ChEIs are in development but have not yet been shown to be active *in vivo* [43].

Finally, therapy with ChEIs may influence neuroinflammation. In experimental studies, activation of the α7 nicotinic receptor by ACh was found to be neuroprotective, at least in neuronal cell cultures exposed to amyloid peptides. The mechanism of action is unclear but may involve entry of Ca^{2+} and effects on APP processing [44]. Aβ is known to bind to α7 nicotinic receptors with high affinity [21]. Moreover, activation of the α7 nicotinic receptor was recently found to attenuate microglial activation, another hallmark of Alzheimer's disease and neurodegenerative diseases [45].

2. Introduction to phenserine

2.1 Cholinesterase inhibitors for the treatment of Alzheimer's disease

In spite of promising developments testing novel targets in psychopharmacology, such as growth factors and cellular or gene therapies, present first-line medications for neurologic and psychiatric diseases most often are aimed at manipulating the action of neurotransmitters. In the field of Alzheimer's disease, it was the early finding of central cholinergic dysfunction that shaped present drug therapy [46]. ChEIs have theoretical advantages over agonist therapies: they only increase signal intensity in synapses that are actually firing and they increase both muscarinic and nicotinic transmission. However, a prerequisite for their cholinergic action is that sufficient residual cholinergic function is present in patient's brains.

The first approved drug for Alzheimer's disease, tacrine (Cognex®), is now obsolete because of a substance-inherent hepatic toxicity. Another allosteric inhibitor of acetylcholinesterase (AChE), donepezil (Aricept®), is presently the most prescribed drug in the US due to its long half-life (once-daily dosing) and relative low adverse-effect profile. Two other ChEIs, rivastigmine (Exelon®) and galantamine (Razadyne®; Reminyl®), were approved by FDA and compete for market shares. Rivastigmine is a pseudo-irreversible inhibitor of both AChE and butyrylcholinesterase (BChE). It produces more prominent effects in the parasympathetic system causing nausea and diarrhea, and it must be dosed by titration according to individual patient tolerability. Its dual inhibition of AChE and BChE may confer an advantage for late-stage Alzheimer's disease [33,47]. Galantamine is an inhibitor of AChE that also has an additional action modulating nicotinic receptors. Several other ChEIs are in development [48]. Interestingly, some ChEIs have been shown to be neuroprotective in neuronal cell culture when excitotoxicity, oxidative stress or amyloid peptides were used as pathogenic factors; their effectiveness may be explained by additional properties such as nicotinic effects (galantamine) or NMDA receptor antagonism (donepezil) [44].

With the present ChEIs on the market, Alzheimer's disease symptoms were found to improve in a subgroup of patients. In clinical trials, ChEI therapy has been found to be effective for Alzheimer's disease for a period of 6 – 24 months [49,50]

(readers are referred to [51] for a differing view). It is a matter of discussion whether ChEIs are only symptomatic treatments by elevating levels of ACh in the brain or if they have additional actions delaying the progression of dementia [52].

Interest in ChEIs is also high for other disease states; recent findings show (for example) that dementia with Lewy bodies is accompanied by a central cholinergic dysfunction that may be more prominent than that in Alzheimer's disease [53]. Down's syndrome also occurs with central cholinergic dysfunction and may be a potential target for ChEI drugs. Finally, other disease states such as brain trauma and delirium may respond favorably to ChEI therapy [52].

2.2 Phenserine: chemistry and biochemistry

Physostigmine (**Figure 1**), the first known ChEI, is a natural, plant-derived compound that has cognitive effects in animals and humans but is not therapeutically used because of its extremely short half-life of ~ 30 – 60 min and prominent parasympathomimetic effects. Several derivatives of physostimine have been developed over the years including heptylphysostigmine (the development of which was discontinued). Phenserine was synthesized in the laboratory of Dr Nigel Greig (National Institute on Ageing). The Greig laboratory has described a series of ChEIs that are based on the structure of physostigmine but show specificity for AChE, BChE or both [54]. Structurally, phenserine is a close relative of physostigmine in which a phenyl group substitutes for the methyl group on the carbamate moiety (**Figure 1**). The synthesis used by Greig and colleagues involved the coupling of eseroline with phenylisocyanate in ether, which was facilitated by addition of sodium [55]. Phenserine was patented for the treatment of cognitive disorders in 1995 together with a description of its effectiveness in a rat experiment involving a 14-unit T-maze [101]. It was again described in the patent literature in 2004 together with selective inhibitors of BChE; in this patent application, its effect on β-APP levels in neuroblastoma cells and in live rats (cerebrospinal fluid [CSF]) were described ([102] and see Section 2.5).

As with physostigmine, phenserine has a chiral center. Whereas physostigmine naturally exists entirely as (-)-physostigmine, phenserine was synthesized in both enantiomeric forms, which have different pharmacologic characteristics. In the literature, phenserine usually denotes (-)-phenserine, whereas (+)-phenserine has been designated as 'posiphen'. (-)-Phenserine was found to inhibit AChE in human erythrocytes with a IC_{50} value of 24 – 45 nM [55,56]. The inhibition type was noncompetitive. Interestingly, the AChE-inhibiting property of phenserine was found to reside exclusively in the (-)-enantiomer as the IC_{50} value for the (+)-enantiomer was 3.5 μM (i.e., 100-fold higher than the racemate) [57]. The IC_{50} value for the inhibition of BChE was reported as 1.56 μM for (-)-phenserine, which, therefore, displays a 65-fold selectivity for AChE over BChE [58]. The (+)-enantiomer lacks BChE activity. Hence (-)-phenserine resembles donepezil (which has a 186-fold selectivity for AChE), whereas rivastigmine is non-selective [52]. Inhibition of BChE is not expected to play a major role in the *in vivo* effects of (-)-phenserine. Phenserine resembles rivastigmine because of the pseudo-irreversible inactivation of AChE by covalent binding that is produced by both drugs.

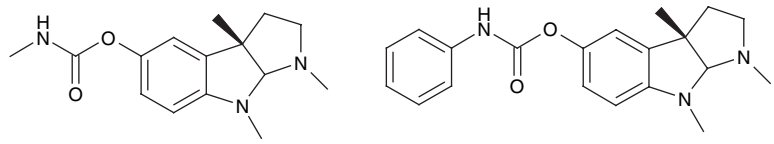
2.3 Phenserine: pharmacokinetics

The pharmacokinetics of (-)-phenserine were investigated in experimental as well as clinical studies. Oral bioavailability was ~ 100%. In rats, the drug reached concentrations in the brain that were 10-fold higher than plasma levels [59]. As an agent that acts by covalent modification of AChE, (-)-phenserine has a much longer duration of action than its plasma half-life (similar to rivastigmine). In preclinical studies, administration of (-)-phenserine caused an inhibition of AChE in blood of > 70%. AChE inhibition declined with a half-life of 8.25 h, whereas the plasma half-life of the drug was 8 – 12 min only. Brain permeability of the compound was demonstrated by microdialysis and by PET studies. The level of extracellular ACh in the striatum, measured by microdialysis, was increased threefold following systemic (-)-phenserine administration [59]. In PET studies in rats, systemic administration of phenserine caused a reduction of the binding of a ^{11}C-labeled muscarinic receptor ligand probably because of a competition between the ligand and increased ACh [60,61]. Thus (-)-phenserine is an effective and brain-permeable inhibitor of AChE.

2.4 Preclinical results in learning and memory tests

Cognitive effects of drugs are generally tested first in animal models that measure learning and memory of rodents in maze paradigms. In rodent models, it is difficult to achieve drug-induced improvements of cognitive function in healthy animals as they routinely perform at high levels in the behavioral tests used. Thus drugs are often tested either in healthy adult animals that were pretreated with cognition-impairing drugs or in elderly animals with reduced cognition. Accordingly, a range of doses of (-)-phenserine (1.5 – 10 mg/kg) were administered to 3-month-old Fischer F-344 rats to assess their ability to overcome the effects of scopolamine (a muscarinic antagonist) 0.75 mg/kg when the rats were tested in a foot-shock-motivated 14-unit T-maze. At these doses, treatment with (-)-phenserine reduced the number of errors and ameliorated runtime, shock frequency and shock duration in this task [62]. A similar effect was observed in a later study after transdermal application of (-)-phenserine; this effect occurred with a parallel inhibition of plasma and brain AChE activity but little action on BChE [63]. The probable mechanism of the effects of (-)-phenserine is inhibition of brain AChE, which is followed by an increase of ACh. Elevated ACh is expected to compete with scopolamine at muscarinic receptors and to increase cholinergic signaling in areas that are relevant for memory (e.g., cortex and hippocampus).

Identical outcomes were observed in another learning paradigm, the Morris water maze, which is a test of spatial learning ability that is strongly dependent on hippocampal function. In this study, scopolamine 1 mg/kg was dosed



A. B.

Figure 1. Chemical structures of (-)-physostigmine (A) and (-)-phenserine (B).

30 min before testing, whereas phenserine was applied at 2 and 4 mg/kg. Scopolamine increased the latency and swimming distance until rats found the platform on four consecutive acquisition trials (once daily) and a probe trial on the fifth day. The higher dose of (-)-phenserine (4 mg/kg) more strongly attenuated the scopolamine-induced deficit than the lower dose (2 mg/kg). (-)-Phenserine 1 mg/kg given without scopolamine did not affect the basal learning curve in 5-month-old rats [66].

(-)-Phenserine was also tested in untreated, aged rats with learning impairments. When given at intermediate doses (1 – 3 mg/kg) for 5 days prior to testing, the drug reduced the number of errors [65]. Adverse side effects were not noted for the doses of 1 and 2 mg/kg.

In the T-maze paradigm, (-)-phenserine was additionally given together with CPP (3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid), an antagonist at glutamatergic NMDA receptors. NMDA receptors are well known to contribute to learning and memory formation and, as expected, CPP 9 mg/kg was found to increase the number of errors in the T-maze. (-)-Phenserine given together with CPP ameliorated the CPP-induced impairment; interestingly, this effect was strongest at the lowest of 3 doses (0.25, 0.5 and 0.75 mg/kg) [66]. Thus although (-)-phenserine antagonized the NMDA receptor blocker-induced effect, it did so at lower doses compared with the muscarinic antagonist. This complex outcome can be rationalized in light of complex interactions of cholinergic signaling and NMDA receptors in the septo-hippocampal pathway [67].

Finally, (-)-phenserine was administered in dogs. Aged Beagle dogs were trained and evaluated for discriminatory behavior. Low-dose scopolamine disrupted working memory but not long-term memory or non-cognitive behavior. Dogs receiving (-)-phenserine showed improved learning and memory compared with placebo [68,69]. The study indicates that dogs respond similarly to (-)-phenserine as rodents.

2.5 Preclinical studies: effects of (-)- and (+)-phenserine on amyloid metabolism

Preclinical studies of phenserine not only described its potency as a ChEI but also revealed a striking influence on the formation of amyloid peptides. As pointed out above, the present discussion on ChEI therapy in dementia not only focuses on tolerability and pharmacokinetics of the drugs but also on targets and functions that these drugs influence in addition to their common target, AChE.

Formation and/or deposition of amyloid peptides may be the underlying cause of Alzheimer's disease (see Section 1). With this in mind, phenserine was tested on parameters of amyloid metabolism in cell cultures and *in vivo* . Earlier findings had indicated that those rats that received lesions of the forebrain cholinergic system respond with an increase of soluble APP in the CSF [70]. It was subsequently reported that scopolamine further increased APP in lesioned rats, whereas treatment with (-)-phenserine decreased APP in CSF [71]. Interestingly, diisopropyl-fluorophosphate, an organophosphate and irreversible inhibitor of AChE, had no effect. In a separate study, cholinergic lesions in the forebrain caused an increased APP expression in the cortex, which correlated with cognitive dysfunction [72].

Further work then focussed on neuronal cell cultures to elucidate the mechanism of action of phenserine. Treatment with both (+)- and (-)-phenserine 5 – 50 µM reduced the expression of APP in neuroblastoma cells and also reduced the formation of total amyloid peptides in the medium of a human neuroblastoma cell line (SK-N-SH). Efficacy was best at 16 h of incubation [73]. APP mRNA levels were unchanged but phenserine reduced expression in a reporter gene assay incorporating a portion of the 5′-untranslated region (5′-UTR) of the *APP* gene that is known to include regulatory elements. It was concluded that phenserine does not affect transcription of *APP* but that it reduces translation of the APP mRNA. *APP* expression is known to be regulated at the level of translation; both IL-1 and TGF-β were found to increase APP expression by a similar mechanism [74,75]. The effect of phenserine on APP translation was independent of its cholinergic activity as both enantiomers have equipotent actions to lower APP [74]. Indeed, it may be mediated by an iron-responsive element in the 5′-UTR of the *APP* gene [73]. In a separate study, phenserine was mimicked by metal chelators such as dimercaptopropanol, desferrioxamine and tetrathiolmolybdate, but also by two unrelated drugs, paroxetine and azithromycin [77]. Thus phenserine may act through an RNA-binding protein that interacts with this area of the 5′-UTR or through metal chelation to suppress APP translation, and it may be more active than some classical chelators due to good bioavailability in the brain. Through one or several – possibly interacting – mechanisms, phenserine lowered APP and Aβ in cell culture and in the brain by ≤ 50 – 60% [78].

Derivatives of phenserine with similar effects and more advantageous therapeutic profiles are actively pursued [79]. Interestingly, the actions of phenserine on amyloid metabolism resemble those of tacrine but are different from those of other ChEIs [76]. For example, donepezil, a prototype ChEI,

was also shown to affect amyloid metabolism but it seems to act via β-secretase and further interactions of individual ChEIs with amyloid metabolism have been suggested (the reader is referred to [36] for further discussion).

What is the significance of this effect? A decrease of APP expression does not necessarily implicate lower formation of amyloid peptides, the probable culprits of APP-related neurotoxicity. However, some studies indicate that stimulation of α-secretase may actually reduce $A\beta_{1-42}$ formation through β- and γ-secretase *in vivo* [80]. If secretase pathways in the brain compete with each other for substrate, then it seems probable that reduction of APP expression may also cause a reduction of amyloid peptide formation. Importantly, overexpression of APP caused by gene duplication is observed both in trisomy 21 (Down's syndrome) and in some familial cases of Alzheimer's disease [81].

2.6 Posiphen

An interesting recent development with phenserine is the focus on (+)-phenserine (posiphen). As described, (+)-phenserine is a poor ChEI but it mimics the action of (-)-phenserine on APP translation. Paradoxically, the lack of ChE inhibition may actually be an advantage for therapeutic purposes because (+)-phenserine largely lacks procholinergic (especially parasympathomimetic) effects and is tolerated in much higher doses than (-)-phenserine (see Section 3 for patient data). In neuroblastoma cells, (+)-phenserine was equally effective as (-)-phenserine with respect to potency (EC_{50} ~ 1 μM) and efficacy (50% maximum reduction of APP translation). (+)-Phenserine reduced APP protein levels in cortex *in vivo* with a ED_{50} of 16 mg/kg (i.e., at a dose level that was much higher than the maximum-tolerated dose of [-]-phenserine) [78]. (+)-Phenserine 35 and 50 mg/kg also reduced β-secretase activity in mouse brain, whereas both (+)- and (-)-phenserine reduced levels of APPs and showed a slight preference for $A\beta_{1-42}$. An unexplained finding is the variability of this effect and the lack of dose dependency, which may be related to metabolism of the compound and formation of active metabolites with different pharmacokinetics [82]. There was also a dose-independent increase of total brain protein in the *in vivo* study that needs further characterization [78].

3. Clinical studies

3.1 Phase I studies

The early clinical development of phenserine is documented by Axonyx Co., a company that developed both enantiomeric forms of phenserine under license from NIH (National Institute on Ageing). As documented on their webpage [201], Phase I clinical studies for (-)-phenserine were performed in 1999 – 2000 in healthy elderly patients [54,83]. Patients tolerated single doses of phenserine 5 and 10 mg (given as tartrate salt) well, but higher doses increased the frequency of headaches and nausea/vomiting [83], a common observation with ChEIs. With respect to pharmacokinetics, C_{max} and AUC values increased with increasing doses but not in a linear fashion. Plasma (-)-phenserine levels and erythrocyte AChE inhibition seemed to correlate closely; the maximum AChE inhibition was 26% (much lower than after intravenous dosing in rats; see Section 2.3). The half-life of AChE inhibition in erythrocytes was 11 h [83].

3.2 Phase II and III studies

A Phase II (proof-of-concept) study was completed in 2001. It was a double-blind, placebo-controlled study in 72 Alzheimer's disease patients; 24 patients received placebo and 48 patients received phenserine 10 mg b.i.d. This dose of the drug was well tolerated. Memory tests showed promising results; significant improvements were noted in a test for short-term memory [56] but the power of the study was inadequate for far-reaching conclusions. A Phase III trial began in 2003 in which Alzheimer's disease patients first received (-)-phenserine 5 mg b.i.d. for 4 weeks and the dose was subsequently increased to 10 mg b.i.d. for a further 4 weeks. One group of patients stayed on this regimen, whereas a randomized group was increased to 15 mg b.i.d. The final doses were continued for the rest of the trial duration (which was 26 weeks). The (-)-phenserine-treated groups displayed higher scores on Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and on Clinically Interview-Based Impression of Change (CIBIC+) throughout the study; however, neither improvement reached statistical significance. A further multicenter Phase III trial was initiated in 2004 by Friedhoff and colleagues (River Vale, USA); however, when the results of the first trial were obtained, this second trial was curtailed. Eventually, 255 patients were finally included in this second trial with a similar treatment regimen as in the first study. The patients from the highest-dose group ([-]-phenserine 15 mg b.i.d.) showed a statistically valid improvement on the ADAS-Cog scale compared with placebo and there was a positive trend on CIBIC+ that did not reach significance [84].

Thus in the clinical studies performed so far, (-)-phenserine showed good tolerability and a positive trend on cognitive measures, which reached significance in a Phase II trial and a curtailed Phase III trial in mild-to-moderate Alzheimer's disease. The lack of a robust response to (-)-phenserine in Phase III studies may well be due to dose restrictions; higher dosing led to more consistent results. Other ChEIs such as tacrine or rivastigmine have to be similarly titrated for optimum effect and effective levels for ChEI inhibition in the brain may not be reached because of adverse side effects observed at suboptimum doses for the inhibition of brain AChE.

The future clinical development plans for (-)-phenserine are unknown at this time; Axonyx has apparently outlicensed the drug to Daewoong Pharmaceutical Co. in Korea. But data on the clinical properties of the compound continue to be reported. A recent abstract revealed that (-)-phenserine 10 – 15 mg/kg reduced the blood plasma level of $A\beta_{1-42}$ (but not $A\beta_{1-40}$) in a small study in healthy volunteers [83].

3.3 Further clinical development

TorreyPines Therapeutics (La Jolla, USA), a company that has superseded Axonyx, has acquired all of its compounds. It is not known at this time whether it will continue to pursue (+)-phenserine (posiphen) as a novel treatment for Alzheimer's disease with the goal to lower amyloid formation in the brain. As mentioned in Section 2.6, (+)-phenserine is a poor inhibitor of AChE and would be expected to have a low cholinergic adverse-effect profile. An IND application for (+)-phenserine was filed in 2005 and a Phase I trial was carried out during 2005 and 2006. Ascending single doses of (+)-phenserine 10 – 160 mg were well tolerated at doses ≤ 80 mg, whereas (+)-phenserine 160 mg caused moderately severe gastrointestinal symptoms including nausea and vomiting [86]. Absorption was rapid within 1 – 2 h and pharmacokinetics seemed to be linear although higher doses of the drug seemed to cause a supralinear increase of plasma levels, which may indicate saturable metabolism. Importantly, plasma levels of (+)-phenserine in humans were equal or greater than those that were effective in mice in reducing brain Aβ levels [87]. No clinical data are available as yet with regards to a Phase II trial.

4. Expert opinion

4.1 Role of cholinergic drugs for future therapy of dementia

In spite of considerable advances in our understanding of pathologic features, the etiology of neurodegenerative diseases has been very difficult to unravel. For example, Alzheimer's disease has some features of accelerated ageing; many of its hallmarks (including amyloid plaques, brain atrophy and cholinergic degeneration) have also been found in very old individuals [88]. Understanding of the disease and why pathological signs occur earlier in Alzheimer's disease populations than in normal elderly is limited by our failure to understand ageing as such. Symptoms and pathologies of the ageing-associated dementias – Alzheimer's disease and Parkinson's disease as well as Lewy body dementia or frontal dementia – can overlap in individual patients. Dementia induced by neurodegeneration can be idopathic or secondary to stroke, traumatic brain injury or cardiovascular disease; and dementia patients often combine signs of neurologic disease with psychiatric symptoms. As a consequence, drugs that show limited effectiveness in specific (e.g., transgenic) animal models of disease may still be clinically useful if they have multiple targets and mechanisms of action.

The present treatment of mild-to-moderate Alzheimer's disease relies on the correction of transmitter deficits by ChEIs. Positive clinical results in late-stage disease have been reported for memantine, a moderate-affinity NMDA receptor blocker, which probably acts as a neuroprotectant. Promising new approaches are aimed at the neuropathological signs of the disease. As a proof of the amyloid hypothesis, it will be important to test specific inhibitors of β- and γ-secretase, the two enzymes that are responsible for amyloid peptide formation. Although these inhibitors were already shown to reduce amyloid load in transgenic mouse models, it is unclear at the present time if reduction of amyloid load will improve cognition in mice and men and what the optimum extent of Aβ reduction might be. An alternative way to get rid of amyloid peptides in the brain would be (active or passive) immunization; experimental and clinical trials are in progress. Many other targets have been proposed for drug development, including neurofibrillary tangles, neuroinflammation and apoptosis [89,90], whereas oxidative stress and glutamatergic toxicity may also play a role. The experimental field suffers from the problem that transgenic mouse models only partially reflect human disease. Moreover, cognition is difficult to assess in mice. We will have to wait for clinical trials as final proof of novel treatment concepts.

Many investigators believe that the formation of amyloid peptides is the causal factor in the progression of Alzheimer's disease and that neuronal cell loss is secondary to amyloid formation and other hallmarks such as τ phosphorylation and lack of axonal NGF transport. However, it must also be kept in mind that a successful treatment of dementias requires the correction of neuronal dysfunctions that are present in patients when diagnosed. In spite of ongoing experimental work, the link between the neuropathological features and neuronal cell loss remains speculative. Cholinergic dysfunction remains the best characterized neuronal impairment and it is difficult at this time to envision an improvement of cognitive function in dementia patients if their cholinergic function is impaired. In other words, although treatment of the cholinergic deficit is clearly not sufficient as such, it is also unlikely that a satisfactory clinical regimen will be developed that excludes drugs targeting cholinergic neurotransmission. Treatment of Alzheimer's disease patients with ChEIs will probably remain a mainstay of therapy for several years to come. In the upcoming years, ChEIs will probably be combined with promising novel drug treatments.

4.2 Phenserine as a drug with multiple actions

Drug therapy with AChE inhibitors was found to be moderately useful for improvement of cognition in Alzheimer's disease; clinical trials in other types of dementia have rarely been performed on a large scale. In addition to cognitive effects, ChEIs were also found to improve psychiatric symptoms in Alzheimer's disease, an effect that may be of similar importance for the clinical use of ChEIs as effects on cognition. As an AChE inhibitor, (-)-phenserine is expected to be similarly useful as other ChEIs. With its high brain:plasma ratio, (-)-phenserine was expected to be well tolerated; however, the clinical trials suggested that phenserine – as with other ChEIs – cannot be dosed for maximum inhibition of brain AChE because adverse peripheral side effects occurred already at moderate doses (> 10 mg); and some adverse cholinergic responses are centrally mediated (e.g., tremor).

However, phenserine has additional actions that make it unique among ChEIs. Although only the (-)-enantiomer is a

potent ChEI, both enantiomers reduce *APP* expression in cell culture and amyloid peptides *in vivo*. The little that we know about *APP* seems to indicate that reduction of *APP* expression may be a beneficial effect. Inflammatory factors as well as free iron are known to upregulate *APP* expression, a process that probably contributes to neurotoxicity and neuronal cell death. Reduction of *APP* expression may also be beneficial because it not only reduces Aβ peptides but also associated *C*-terminal fragments that may be neurotoxic [91]. In addition, (+)-phenserine has recently been reported to stimulate human stem cells growing in mice [92]. Ongoing research may reveal additional properties of this interesting molecule.

From these considerations, the most promising aspect of phenserine may be a combination of its enantiomers to act as dual-action drugs. A small dose of (-)-phenserine (the ChEI) may be combined with a much larger dose of (+)-phenserine (the non-ChEI, which also influences APP metabolism). Long-term effects of this combination may be an improvement of cholinergic transmission, reduction of amyloid peptide formation and potentially anti-inflammatory and/or neurotrophic actions. Different modes of applications can be envisioned for this drug (e.g., nasal or transdermal routes that were already suggested in the patent literature). However, long-term clinical studies will be required to evaluate these putative benefits.

Disclosure

The author has no conflict of interest to declare and no fee has been received for preparation of the manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. HARDY J, SELKOE DJ: The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* (2002) **297**:353-356.
2. NEVE RL, ROBAKIS NK: Alzheimer's disease: a re-examination of the amyloid hypothesis. *Trends Neurosci.* (1998) **21**:15-19.
3. WALSH DM, SELKOE DJ: Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* (2004) **44**:181-193.
- • **Review covering many good arguments for the amyloid hypothesis of AD.**
4. NASLUND J, HAROUTUNIAN V, MOHS R *et al.*: Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. *JAMA* (2000) **283**:1571-1577.
5. KLEIN WL, KRAFFT GA, FINCH CE: Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum? *Trends Neurosci.* (2001) **24**:219-224.
6. SMALL DH, MOK SS, BORNSTEIN JC: Alzheimer's disease and Aβ toxicity: from top to bottom. *Nat. Rev. Neurosci.* (2001) **2**:595-598.
7. WIRTHS O, MULTHAUP G, BAYER TA: A modified β-amyloid hypothesis: intraneuronal accumulation of the β-amyloid peptide – the first step of a fatal cascade. *J. Neurochem.* (2004) **91**:513-520.
8. CHAPMAN PF, FALINSKA AM, KNEVETT SG, RAMSAY MF: Genes, models and Alzheimer's disease. *Trends Genet.* (2001) **17**:254-261.
9. ODDO S, CACCAMO A, SHEPHERD JD *et al.*: Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* (2003) **39**:409-421.
10. SHINOE T, MATSUI M, TAKETO MM, MANABE T: Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. *J. Neurosci.* (2005) **25**:11194-11200.
11. WILLEM M, GARRATT AN, NOVAK B *et al.*: Control of peripheral nerve myelination by the β-secretase BACE1. *Science* (2006) **314**:664-666.
12. FRANCIS PT, PALMER AM, SNAPE M, WILCOCK GK: The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J. Neurol. Neurosurg. Psychiatry* (1999) **66**:137-147.
13. SARTER M, GEHRING WJ, KOZAK R: More attention must be paid: the neurobiology of attentional effort. *Brain Res. Rev.* (2006) **51**:145-160.
14. MESULAM M: The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn. Mem.* (2004) **11**:43-49.
- • **Good summary of arguments for and against the relevance of cholinergic dysfunction in Alzheimer's disease.**
15. COLLERTON D: Cholinergic function and intellectual decline in Alzheimer's disease. *Neuroscience* (1986) **19**:1-28.
16. DONG H, CSERNANSKY CA, MARTIN MV, BERTCHUME A, VALLERA D, CSERENANSKY JG: Acetylcholinesterase inhibitors ameliorate behavioural deficits in the Tg2576 mouse model of Alzheimer's disease. *Psychopharmacology* (2005) **181**:145-152.
17. CAPSONI S, UGOLINI G, COMPARINI A, RUBERTI F, BERARDI N, CATTANEO A: Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. *Proc. Natl. Acad. Sci. USA* (2000) **97**:6826-6831.
18. TUSZYNSKI MH, THAL L, PAY M *et al.*: A Phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat. Med.* (2005) **11**:551-555.
19. SALEHI A, DELCROIX JD, BELICHENKO PV *et al.*: Increased APP expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron* (2006) **51**:29-42.
20. KLEIN J: Membrane breakdown in acute and chronic neurodegeneration: focus on choline-containing phospholipids. *J. Neural Transm.* (2000) **107**:1027-1063.
21. AULD DS, KORNECOOK TJ, BASTIANETTO S, QUIRION R: Alzheimer's disease and the basal forebrain cholinergic system: relations to β-amyloid peptides, cognition, and treatment strategies. *Prog. Neurobiol.* (2002) **68**:209-245.
- • **Summarizes many mechanisms by which amyloid could damage cholinergic systems.**
22. YAMADA K, NABESHIMA T: Animal models of Alzheimer's disease and evaluation of anti-dementia drugs. *Pharmacol. Ther.* (2000) **88**:93-113.
23. CHOI SH, PARK CH, KOO JW *et al.*: Memory impairment and cholinergic dysfunction by centrally administered

Aβ and carboxyl-terminal fragment of Alzheimer's APP in mice. *FASEB J.* (2001) **15**:1816-1818.

24. GAU JT, STEINHILB ML, KAO TC *et al.*: Stable β-secretase activity and presynaptic cholinergic markers during progressive central nervous system amyloidogenesis in Tg2576 mice. *Am. J. Pathol.* (2002) **160**:731-738.
25. HARTMANN J, ERB C, EBERT U *et al.*: Central cholinergic functions in human amyloid precursor protein knock-in/presenilin-1 transgenic mice. *Neuroscience* (2004) **125**:1009-1017.
26. BELLUCCI A, LUCCARINI I, SCALI C *et al.*: Cholinergic dysfunction, neuronal damage and axonal loss in TgCRND8 mice. *Neurobiol. Dis.* (2006) **23**:260-272.
27. BALES KR, TZAVARA ET, WU S *et al.*: Cholinergic dysfunction in a mouse model of Alzheimer disease is reversed by an anti-A β antibody. *J. Clin. Invest.* (2006) **116**:825-832.
    - **Makes a strong argument that reducing amyloid peptides may alleviate the cholinergic deficit.**
28. CIRRITO JR, MAY PC, O'DELL MA *et al.*: *In vivo* assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-β metabolism and half-life. *J. Neurosci.* (2002) **23**:8844-8853.
29. TSANG SW, LAI MK, KIRVELL S *et al.*: Impaired coupling of M1 muscarinic receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease. *Neurobiol. Aging* (2006) **27**:1216-1223.
30. BELL KF, DUCATENZEILER A, RIBEIRO-DA-SILVA A, DUFF K, BENNETT DA, CUELLO AC: The amyloid pathology progresses in a neurotransmitter-specific manner. *Neurobiol. Aging* (2006) **27**:1644-1657.
    - **Shows that cholinergic neurons may be more susceptible than other neurons in early stages of amyloid deposition.**
31. GERMAN DC, YAZDANI U, SPECIALE SG, PASBAKHSH P, GAMES D, LIANG CL: Cholinergic neuropathology in a mouse model of Alzheimer's disease. *J. Comp. Neurol.* (2003) **462**:371-381.
32. ERB C, TROOST J, KOPF S *et al.*: Compensatory mechanisms enhance hippocampal acetylcholine release in transgenic mice expressing human acetylcholinesterase. *J. Neurochem.* (2001) **77**:638-646.
33. HARTMANN J, KIEWERT C, DUYSEN EG, LOCKRIDGE O, GREIG NH, KLEIN J: Excessive hippocampal acetylcholine levels in acetylcholinesterase-deficient mice are moderated by butyrylcholinesterase activity. *J. Neurochem.* (2007) **100**:1421-1429.
    - **Suggests that inhibition of BChE may be beneficial when AChE activity is low.**
34. DEKOSKY ST, IKONOMOVIC MD, STYREN SD *et al.*: Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann. Neurol.* (2002) **51**:145-155.
35. NITSCH RM, SLACK BE, WURTMAN RJ, GROWDON JH: Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* (1992) **258**:304-307.
36. RACCHI M, MAZZUCCHELLI M, LENZKEN SC, PORRELLO E, LANNI C, GOVONI S: Role of acetylcholinesterase inhibitors in the regulation of amyloid β precursor protein (AβPP) metabolism. *Chem. Biol. Interact.* (2005) **157-158**:335-338.
    - **Summarizes effects of cholinesterase inhibitors on amyloid metabolism.**
37. ROBERSON MR, HARRELL LE: Cholinergic activity and amyloid precursor protein metabolism. *Brain Res. Rev.* (1997) **25**:50-69.
38. NI Y, ZHAO X, BAO G *et al.*: Activation of β2-adrenergic receptor stimulates γ-secretase activity and accelerates amyloid plaque formation. *Nat. Med.* (2006) **12**:1390-1396.
39. MESHORER E, SOREQ H: Virtues and woes of AChE alternative splicing in stress-related neuropathologies. *Trends Neurosci.* (2006) **29**:216-224.
40. INESTROSA NC, ALVAREZ A, PEREZ CA *et al.*: Acetylcholinesterase accelerates assembly of amyloid-β-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* (1996) **16**:881-891.
41. REES T, HAMMOND PI, SOREQ H, YOUNKIN S, BRIMIJOIN S: Acetylcholinesterase promotes β-amyloid plaques in cerebral cortex. *Neurobiol. Aging* (2003) **24**:777-787.
42. BARTOLINI M, BERTUCCI C, CAVRINI V, ANDRISANO V: β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem. Pharmacol.* (2003) **65**:407-416.
43. CASTRO A, MARTINEZ A: Targeting β-amyloid pathogenesis through acetylcholinesterase inhibitors. *Curr. Pharm. Des.* (2006) **12**:4377-4387.
44. FRANCIS PT, NORDBERG A, ARNOLD SE: A preclinical view of cholinesterase inhibitors in neuroprotection: do they provide more than symptomatic benefits in Alzheimer's disease? *Trends Pharmacol. Sci.* (2005) **26**:104-111.
45. SHYTLE RD, MORI T, TOWNSEND K *et al.*: Cholinergic modulation of microglial activation by α7 nicotinic receptors. *J. Neurochem.* (2004) **89**:337-343.
46. PALMER AM: Pharmacotherapy for Alzheimer's disease: progress and prospects. *Trends Pharmacol. Sci.* (2002) **23**:426-433.
47. BALLARD CG, GREIG NH, GUILLOZET-BONGAARTS AL, ENZ A, DARVESH S: Cholinesterases: roles in the brain during health and disease. *Curr. Alzheimer Res.* (2005) **2**:307-318.
48. MARTINEZ A, CASTRO A: Novel cholinesterase inhibitors as future effective drugs for the treatment of Alzheimer's disease. *Expert Opin. Investig. Drugs* (2006) **15**:1-12.
49. WILKINSON DG, FRANCIS PT, SCHWAM E, PAYNE-PARRISH J: Cholinesterase inhibitors used in the treatment of Alzheimer's disease: the relationship between pharmacological effects and clinical efficacy. *Drugs Aging* (2004) **21**:453-478.
50. BIRKS J: Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst. Rev.* (2006):CD005593.
51. KADUSZKIEWICZ H, ZIMMERMANN T, BECK-BORNHOLDT HP, VAN DEN BH: Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. *BMJ* (2005) **331**:321-327.
52. GIACOBINI E: Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacol. Res.* (2004) **50**:433-440.
    - **Discusses cholinesterase inhibitors and their potential use in various neurologic diseases.**

53. AARSLAND D, MOSIMANN UP, MCKEITH IG: Role of cholinesterase inhibitors in Parkinson's disease and dementia with Lewy bodies. *J. Geriatr. Psychiatry Neurol.* (2004) **17**:164-171.

54. GREIG NH, SAMBAMURTI K, YU QS, BROSSI A, BRUINSMA GB, LAHIRI DK: An overview of phenserine tartrate, a novel acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Curr. Alzheimer Res.* (2005) **2**:281-290.

55. YU Q, HOLLOWAY HW, FLIPPEN-ANDERSON JL, HOFFMAN B, BROSSI A, GREIG NH: Methyl analogues of the experimental Alzheimer drug phenserine: synthesis and structure/activity relationships for acetyl- and butyrylcholinesterase inhibitory action. *J. Med. Chem.* (2001) **44**:4062-4071.

56. AL JAFARI AA, KAMAL MA, GREIG NH, ALHOMIDA AS, PERRY ER: Kinetics of human erythrocyte acetylcholinesterase inhibition by a novel derivative of physostigmine: phenserine. *Biochem. Biophys. Res. Commun.* (1998) **248**:180-185.

57. YU QS, PEI XF, HOLLOWAY HW, GREIG NH, BROSSI A: Total syntheses and anticholinesterase activities of (3aS)-N(8)-norphysostigmine, (3aS)-N(8)-norphenserine, their antipodal isomers, and other N(8)-substituted analogues. *J. Med. Chem.* (1997) **40**:2895-2901.

58. YU Q, HOLLOWAY HW, UTSUKI T, BROSSI A, GREIG NH: Synthesis of novel phenserine-based-selective inhibitors of butyrylcholinesterase for Alzheimer's disease. *J. Med. Chem.* (1999) **42**:1855-1861.

59. GREIG NH, DE MICHELI E, HOLLOWAY HW *et al.*: The experimental Alzheimer drug phenserine: preclinical pharmacokinetics and pharmacodynamics. *Acta Neurol. Scand. Suppl.* (2000) **176**:74-84.

60. SKADDAN MB, KILBOURN MR, SNYDER SE, SHERMAN PS: Acetylcholinesterase inhibition increases *in vivo N*-(2-[18F]fluoroethyl)-4-piperidyl benzilate binding to muscarinic acetylcholine receptors. *J. Cereb. Blood Flow Metab.* (2001) **21**:144-148.

61. SKADDAN MB, JEWETT DM, SHERMAN PS, KILBOURN MR: (R)-*N*-[11C]methyl-3-pyrrolidyl benzilate, a high-affinity reversible radioligand for PET studies of the muscarinic acetylcholine receptor. *Synapse* (2002) **45**:31-37.

62. IIJIMA S, GREIG NH, GAROFALO P *et al.*: Phenserine: a physostigmine derivative that is a long-acting inhibitor of cholinesterase and demonstrates a wide dose range for attenuating a scopolamine-induced learning impairment of rats in a 14-unit T-maze. *Psychopharmacology* (1993) **112**:415-420.

63. UTSUKI T, UCHIMURA N, IRIKURA M *et al.*: Pre-clinical investigation of the topical administration of phenserine: transdermal flux, cholinesterase inhibition and cognitive efficacy. *J. Pharmacol. Exp. Ther.* (2007) **321**(1):353-361.

•• **Recent study on cognitive effects of phenserine after topical administration.**

64. JANAS AM, CUNNINGHAM SC, DUFFY KB *et al.*: The cholinesterase inhibitor, phenserine, improves Morris water maze performance of scopolamine-treated rats. *Life Sci.* (2005) **76**:1073-1081.

65. IKARI H, SPANGLER EL, GREIG NH *et al.*: Maze learning in aged rats is enhanced by phenserine, a novel anticholinesterase. *Neuroreport* (1995) **6**:481-484.

66. PATEL N, SPANGLER EL, GREIG NH, YU QS, INGRAM DK, MEYER RC: Phenserine, a novel acetylcholinesterase inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the *N*-methyl-D-aspartate receptor. *Neuroreport* (1998) **9**:171-176.

67. MOOR E, AUTH F, DEBOER P, WESTERINK BH: Septal and hippocampal glutamate receptors modulate the output of acetylcholine in hippocampus: a microdialysis study. *J. Neurochem.* (1996) **67**:310-316.

68. ARAUJO JA, STUDZINSKI CM, MILGRAM NW: Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. *Prog. Neuropsychopharmacol. Biol. Psychiatry* (2005) **29**:411-422.

69. STUDZINSKI CM, ARAUJO JA, MILGRAM NW: The canine model of human cognitive aging and dementia: pharmacological validity of the model for assessment of human cognitive-enhancing drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry* (2005) **29**:489-498.

70. WALLACE WC, LIEBERBURG I, SCHENK D, VIGO-PELFREY C, DAVIS KL, HAROUTUNIAN V: Chronic elevation of secreted amyloid precursor protein in subcortically lesioned rats, and its exacerbation in aged rats. *J. Neurosci.* (1995) **15**:4896-4905.

71. HAROUTUNIAN V, GREIG N, PEI XF *et al.*: Pharmacological modulation of Alzheimer's β-amyloid precursor protein levels in the CSF of rats with forebrain cholinergic system lesions. *Brain Res. Mol. Brain Res.* (1997) **46**:161-168.

72. LIN L, GEORGIEVSKA B, MATTSSON, ISACSON O: Cognitive changes and modified processing of amyloid precursor protein in the cortical and hippocampal system after cholinergic synapse loss and muscarinic receptor activation. *Proc. Natl. Acad. Sci. USA.* (1999) **96**:12108-12113.

73. SHAW KT, UTSUKI T, ROGERS J *et al.*: Phenserine regulates translation of β -amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. *Proc. Natl. Acad. Sci. USA* (2001) **98**:7605-7610.

•• **Describes mechanism of action of the effect of phenserine on APP expression.**

74. ROGERS JT, LEITER LM, MCPHEE J *et al.*: Translation of the alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5′-untranslated region sequences. *J. Biol. Chem.* (1999) **274**:6421-6431.

75. LAHIRI DK, CHEN D, VIVIEN D, GE YW, GREIG NH, ROGERS JT: Role of cytokines in the gene expression of amyloid β-protein precursor: identification of a 5′-UTR-binding nuclear factor and its implications in Alzheimer's disease. *J. Alzheimers Dis.* (2003) **5**:81-90.

76. LAHIRI DK, FARLOW MR, HINTZ N, UTSUKI T, GREIG NH: Cholinesterase inhibitors, β-amyloid precursor protein and amyloid β-peptides in Alzheimer's disease. *Acta Neurol. Scand. Suppl.* (2000) **176**:60-67.

77. MORSE LJ, PAYTON SM, CUNY GD, ROGERS JT: FDA-preapproved drugs targeted to the translational regulation and processing of the amyloid precursor protein. *J. Mol. Neurosci.* (2004) **24**:129-136.

78. LAHIRI DK, CHEN D, MALONEY B *et al.*: The experimental Alzheimer's disease drug posiphen [(+)-phenserine] lowers amyloid-β peptide levels in cell culture and mice. *J. Pharmacol. Exp. Ther.* (2007) **320**:386-396.

•• **Characterizes effects of (+)-phenserine on amyloid peptides *in vitro* and *in vivo*.**

79. UTSUKI T, YU QS, DAVIDSON D *et al.*: Identification of novel small molecule inhibitors of amyloid precursor protein synthesis as a route to lower Alzheimer's disease amyloid-β peptide. *J. Pharmacol. Exp. Ther.* (2006) **318**:855-862.

80. HARTMANN T, BIEGER SC, BRUHL B *et al.*: Distinct sites of intracellular production for Alzheimer's disease A β40/42 amyloid peptides. *Nat. Med.* (1997) **3**:1016-1020.

81. ROVELET-LECRUX A, HANNEQUIN D, RAUX G *et al.*: APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat. Genet.* (2006) **38**:24-26.

- **Recent paper showing that APP overexpression has pathologic consequences.**

82. YU QS, LUO W, HOLLOWAY HW *et al.*: Racemic N^1-norphenserine and its enantiomers: unpredicted inhibition of acetyl- and butyrylcholinesterase and β-amyloid precursor protein *in vitro*. *Heterocycles* (2003) **61**:529-539.

83. GREIG NH, RUCKLE J, COMER P *et al.*: Anticholinesterase and pharmacokinetic profile of phenserine in healthy elderly individuals. *Curr. Alzheimer Res.* (2005) **2**:483-492.

- **Phase I study of (-)-phenserine in healthy volunteers.**

84. WINBLAD B, GIACOBINI E, BRUINSMA G, WALTERS E, FRIEDHOFF L: Double-blind placebo-controlled evaluation of the safety and efficacy of phenserine tartrate for the treatment of mild to moderate Alzheimer's disease. *9th International Geneva/Springfield Symposium on Advances in Alzheimer's disease*, Geneva, Switzerland (2006):11A (Abstract).

85. SAMBAMURTI K, ANNAMALAI P, ANITHA S *et al.*: An acetylcholinesterase inhibitor, phenserine, reduces Aβ1-42 in healthy volunteers. *Society for Neuroscience Conference*, Atlanta, Georgia, USA (2006):120.11 (Abstract).

86. CULLEN EI, WALTERS EJ, FRIEDHOFF LT, BRUINSMA GB: A single ascending dose study of the pharmacokinetics and tolerability of posiphen in healthy men and women. *Society for Neuroscience Conference*, Atlanta, Georgia (USA) (2006):412.10 (Abstract).

87. CULLEN E, BRUINSMA G, GREIG NH, FRIEDHOFF L, CHEN D, LAHIRI DK: Brain β-amyloid (1-42) in mice treated orally with posiphen tartrate is significantly lower than in vehicle controls. *9th International Geneva/Springfield Symposium on Advances in Alzheimer's disease*, Geneva, Switzerland (2006):12A (Abstract).

88. BEACH TG, HONER WG, HUGHES LH: Cholinergic fibre loss associated with diffuse plaques in the non-demented elderly: the preclinical stage of Alzheimer's disease? *Acta Neuropathol.* (1997) **93**:146-153.

89. CUMMINGS JL: Alzheimer's disease. *New Engl. J. Med.* (2004) **351**:56-67.

90. MATTSON MP: Pathways towards and away from Alzheimer's disease? *Nature* (2004) **430**:631-639.

91. SAMBAMURTI K, SURAM A, VENUGOPAL C *et al.*: A partial failure of membrane protein turnover may cause Alzheimer's disease: a new hypothesis. *Curr. Alzheimer Res.* (2006) **3**:81-90.

- **Presents 'traffic jam' hypothesis linking APP overexpression and Alzheimer's disease.**

92. OHMITSU M, MARUTLE A, NILBRATT M, GREIG N, NORDBERG A, SUGAYA K: Effect of (+)-phenserine treatment in APP23 TG mice transplanted with human neural stem cells. *Society for Neuroscience Conference*, Atlanta, Georgia, USA (2006):417.5/A5 (Abstract).

Patents

101. GREIG *ET AL.*: US5409948.

102. GREIG *ET AL.*: US6683105B2.

Website

201. http://www.axonyx.com
Axonyx website
(Accessed December 2006).

Affiliation

Jochen Klein PhD
†Address for correspondence
†[1]University of Frankfurt College of Pharmacy,
Biocenter N260, Max-von-Laue-Str. 9, D-60438
Frankfurt, Germany
Tel: +69 798 29366;
Fax: +69 798 29374;
E-mail: Klein@em.uni-frankfurt.de
[2]TTUHSC School of Pharmacy,
Department of Pharmaceutical Sciences,
1300 Coulter Drive,
Amarillo, TX 79106, USA