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European Journal of Pharmacology 393 (2000) 255–263

ejp

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Modulation of β-amyloid precursor protein processing and tau phosphorylation by acetylcholine receptors

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Accepted 21 January 2000

Abstract

Neurofibrillary lesions and senile plaques that are composed mainly of hyperphosphorylated tau protein and the amyloid-β peptide derived from the amyloid precursor protein, respectively, are classical hallmarks of Alzheimer's disease. A number of studies strongly suggests that amyloid-β formation and amyloid depositions are linked to the pathogenesis of Alzheimer's disease. Recent findings suggest that very low concentrations of the amyloid-β can inhibit various cholinergic neurotransmitter functions independently of apparent neurotoxicity. Many factors have been shown to influence the processing of amyloid precursor protein, including activation of muscarinic and nicotinic receptors. This review focus on some recent studies concerning the regulation of amyloid precursor protein processing and modulation of tau phosphorylation by acetylcholine receptor stimulation and how cholinergic deficits and amyloid-β might be related to one another.

Keywords: Alzheimer's disease; Amyloid precursor protein; Amyloid-β peptide; Plaque; Tau; Tangle; Nicotine; Muscarinic receptor; Nicotinic receptor; Cholinesterase inhibitor

1. Introduction

Senile plaques and neurofibrillary lesions are the major histopathological hallmarks of Alzheimer's disease. Plaques are composed mainly of a small insoluble peptide of 40–42 amino acids called amyloid-β. Several lines of evidence suggest that increased production and deposition of the amyloid-β contributes to the pathogenesis of Alzheimer's disease (reviewed in Selkoe, 1996, 1999; Hardy, 1997; Sabbagh et al., 1997). Amyloid-β is derived by proteolytic processing from a larger glycosylated membrane-bound protein, the amyloid β-precursor protein (APP). Cleavage of APP can occur via several pathways that are either amyloidogenic, generating amyloid-β, or non-amyloidogenic, generating soluble APP and p3, a truncated amyloid-β fragment (Fig. 1). The biology of APP and amyloid-β and processing of APP has been extensively reviewed elsewhere (Checler, 1995; Mattson, 1997; Mills and Reiner, 1999). The major route of APP processing is by α-secretase, an enzyme that cleaves within the amyloid-β sequence thereby preventing the formation of amyloid-β. Cleavage of APP by β and γ-secretase at the N and C-terminal ends of amyloid-β respectively generates soluble APP and amyloid-β. Besides the secretory processing pathway full-length APP can be target to the endosomal system (Haass et al., 1992), endoplasmic reticulum or Golgi complex (Cook et al., 1997; Hartmann et al. 1997). The major form of amyloid-β, roughly 90% of the total secreted amyloid-β, contains 40 amino acids, amyloid-β-(1–40), whereas amyloid-β-(1–42) is produced in much lower quantities. However, the amyloid-β-(1–42) aggregates far more rapidly into amyloid fibrils than does amyloid-β-(1–40). Evidence for neurotrophic functions of soluble APP in the nervous system exists, including promotion of neuronal cell survival, adhesive interactions, neurite outgrowth, synaptogenesis and synaptic plasticity (for reviews see Small and McLean, 1999; Mattson, 1997). Processing of APP can be regulated by a variety of factors that include stimulation of receptors for acetylcholine, serotonin, glutamate and neuropeptides (Nitsch and Growdon, 1994).

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00028-5

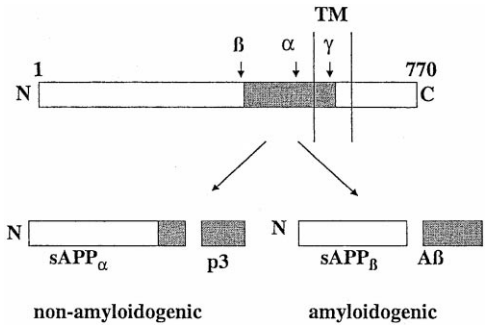

Fig. 1. Secretase cleavage sites of APP and pathways of APP processing. APP is processed via two alternative pathways, resulting in a cleavage by α- or β-secretase generating soluble APPα or APPβ, respectively. The remaining C-terminal fragment in the membrane can undergo cleavage by γ-secretase to generate either p3 when following α-secretase cleavage or amyloid-β when following β-secretase cleavage. A single transmembrane-spanning domain (TM) is indicated by the vertical lines.

Another feature of Alzheimer's disease is the widespread degeneration and dysfunction of the basal-forebrain cholinergic system in brain. Cholinergic pathways serve important functions in the processes of learning and memory and cholinergic neurotransmission is impaired in Alzheimer's disease. Drug development for treatment of Alzheimer's disease has generally targeted enhancement of the cholinergic system via cholinergic agonists (muscarinic and nicotinic) or cholinesterase inhibitors which indirectly increase the synaptic concentration of acetylcholine (Holladay et al., 1997; Newhouse et al., 1997; Nordberg and Svensson, 1998). A consistent loss of nicotinic receptors in the cerebral cortex and hippocampus in Alzheimer brain has been reported, whereas most studies suggest that there are no changes in the number of muscarinic receptors between Alzheimer and control brains (for review see Nordberg, 1992; Pavia et al., 1998). Although the muscarinic receptor density is not changed, functional studies of muscarinic M_1 receptor-mediated signal transduction have shown a loss of receptor-G protein coupling and other impairments in the Alzheimer brain (Ladner and Lee, 1998; Pavia et al., 1998). This review will briefly discuss the involvement of muscarinic and nicotinic receptors on the processing of APP, production of amyloid-β and regulation of tau protein phosphorylation and the potential relevance of this relationship to the cholinergic deficits in Alzheimer's disease (Table 1).

2. Cholinergic activity and amyloid precursor protein processing

2.1. Muscarinic receptor and APP processing

The first study demonstrating that processing of APP could be regulated by cell surface receptors involved human kidney embryonic cells overexpressing muscarinic M_1 and M_3 receptors (Nitsch et al., 1992). Activation of these receptors with the muscarinic receptors agonist carbachol significantly increased the release of soluble APP. Later studies extended these initial findings in a variety of cell lines (Buxbaum et al., 1992, 1994; Hung et al., 1993; Wolf et al., 1995). In an attempt to study receptor coupled regulation of APP processing in brain tissue, hippocampal slices from rat brain were electrically stimulated (Nitsch et al., 1993). The depolarisation induced an increase in soluble APP secretion that was blocked by tetrotoxin, indicating that this release resulted from formation of action potentials and that APP processing may be regulated by neuronal activity in brain. Muscarinic M_1 and M_3 receptor agonists stimulated soluble APP release from cortical slices in a dose-dependent manner. Pittel et al. (1996) also found that secretion of soluble APP from rat cerebral cortex was increased by carbachol and AF102B (muscarinic M_1 receptor agonist) while secretion from slices of cerebellum, a brain region that is very poor in muscarinic M_1 receptors, was enhanced only by carbachol. Not only have an increase in the secretion of soluble APP been seen but stimulation of muscarinic M_1 receptor transfected cells by carbachol also reduced the secretion of amyloid-β into the medium (Buxbaum et al., 1993; Hung et al., 1993; Wolf et

Table 1
Effects of various agents on phosphorylation of tau protein

Agent	Spectrum of action	Cell system	Phosphorylation	Dephosphorylation	Reference
Carbachol	Muscarinic receptor	M_1 transfected	decrease	increase	Sadot et al., 1996
AF 102B	agonists	PC 12 cells			
AF150S		APO E deficient mice	decrease		Genis et al., 1999
Nicotine	Nicotinic receptor	SH-SY5Y cells	increase	increase	Hellström-Lindahl et al., 2000
Epibatidine	agonists				
Tacrine	Cholinesterase	SH-SY5Y cells	increase	increase	Hellström-Lindahl et al., 2000
Donepezil	inhibitors				
Galanthamine					
Glutamate	Excitatory transmitter	Hippocampal neurons	increase		Mattson, 1990
		Cortical neurons	increase		Sindou et al., 1994
A23187	Calcium	Hippocampal neurons	increase		Mattson, 1990
	ionophore	SH-SY5Y cells	increase	decrease	Shea et al., 1997

al., 1995). Although an increased release of soluble APP is expected to be accompanied by a decrease in amyloid-β secretion, this is not always true (Mills and Reiner, 1999). In human neuroblastoma cells amyloid-β production was not changed by protein kinase C activation whereas secretion of soluble APP was stimulated (Dyrks et al., 1994).

G-protein coupled receptor stimulation of soluble APP release appears to be selective for receptors subtypes that are coupled to the protein kinase C dependent signalling pathway. Activation of cells expressing the muscarinic M_2 and M_4 receptor subtype that are linked to the cAMP signal transduction pathway does not change soluble APP release (Nitsch et al., 1992). Interestingly, stimulation of other G-protein coupled receptors linked to phosphotidylinositol hydrolysis could also increase soluble APP secretion, as has been shown for vasopressin and bradykinin (Nitsch et al., 1998), 5-HT receptors (Nitsch et al., 1996) and interleukin 1 receptors (Buxbaum et al., 1992). Further support for a role for protein kinase C signalling pathway in regulating APP processing comes from studies where direct activation of protein kinase C by phorbol esters has been shown to coincidentally increase soluble APP and decrease amyloid-β release (reviewed in Gandy and Greengard, 1994; Mills and Reiner, 1999). Moreover, these effects were blocked by various protein kinase C inhibitors. Conversely, phosphate inhibitors promote soluble APP release (Slack et al., 1995). The downstream phosphorylation target of protein kinase C activity with respect to the mechanism of soluble APP secretion is still unclear, but may include additional kinase steps. It is possible that substrates other than APP, e.g., proteases involved in APP cleavage, are phosphorylated in response to receptor activation (Nitsch et al., 1994).

Taken together, these findings show that muscarinic receptors in brain can regulate APP processing pathways and activation of these receptors can stimulate non-amyloidogenic α-secretase processing of APP and in many cases concurrently inhibit the β-secretase processing pathway.

2.2. Nicotinic receptors and APP processing

Unlike G-protein-coupled receptors, few studies have examined regulation of soluble APP release by activation of ligand-gated channels such as nicotinic receptors. Whether nicotine modulates the expression and processing of APP was studied in PC 12 cells (Kim et al., 1997). Treatment with nicotine at concentrations > 50 μM significantly increased the release of soluble APP into the medium without affecting the expression of APP mRNA. The effect of nicotine on the secretion of soluble APP was attenuated by co-treatment with the nicotinic receptor antagonist mecamylamine, indicating that nicotine could enhance release of soluble APP through specific interactions with nicotinic receptors. In PC12 cells transiently transfected with the Swedish APP mutation, nicotine could not reduce amyloid-β production whereas nicotine could stimulate the release of soluble APP (Kim et al., 1997). The effect of nicotine on soluble APP secretion was recently confirmed by Kuisak et al. (1999) in stably transfected PC12 cells expressing the human wildtype and mutant APP. The increase of soluble APP was inhibited by the α7 nicotinic receptor antagonist methyllycaconitine, or EGTA, a calcium chelator. Furthermore, the increased Ca^{2+} influx induced by nicotine correlated with the increase in soluble APP secretion, suggesting that Ca^{2+} influx through nicotinic receptors is sufficient to enhance this secretion. This is in agreement with the findings from several studies indicating that Ca^{2+} can regulate APP processing (Loeffler and Huber, 1993; Buxbaum et al., 1994; Querfurth and Selkoe, 1994). However, there are also reports where Ca^{2+} did not increase soluble APP release (Nitsch et al.,1992, 1996). In bovine chromaffin cell cultures, nicotine, carbachol and potassium-induced depolarization stimulated secretion of soluble full-length APP (Efthimiopoulos et al., 1996). In Alzheimer brain, the density of nicotinic receptors is consistently reduced, which may lead to an aberrant processing of APP. Reduced levels of soluble APP may secondary contribute to the neuronal loss in Alzheimer's disease.

2.3. Cholinesterase inhibitors and APP processing

Among new treatment strategies in Alzheimer's disease, the cholinesterase inhibitors have so far been most promising in improving cognitive function of Alzheimer patients. In addition to acting as an inhibitor of cholinesterase, several of these drugs have been shown to have other pharmacological actions. These effects include up-regulation of nicotinic receptors in vitro and in vivo (Nordberg et al., 1992; Svensson and Nordberg, 1996), interactions with an allosteric site on the nicotinic receptor that is distinct from that for acetylcholine (Pereira et al., 1994; Maelicke et al., 1995; Svensson and Nordberg, 1997), modulation of tau protein phosphorylation in cells (Hellström-Lindahl et al., 2000) and attenuation of amyloid-β-(25–35) toxicity in PC 12 cells (Svensson and Nordberg, 1998). Cholinesterase inhibitors have been shown to alter the release of soluble APP in rat cortex and in various cell lines. Physostigmine, heptl-physostigmine, and 2,2-dichlorovinyldimethyl phosphate elevated soluble APP secretion in rat cortical slices (Mori et al., 1995). Lahiri et al. (1994) reported that normal levels of secretion of soluble APP by glial, fibroblast, PC12 and neuroblastoma cells were severely inhibited after treatment with tacrine. Later, this group showed that tacrine treatment of human neuroblastoma cells reduced the levels of total amyloid-β, amyloid-β-(1–40) and amyloid-β-(1–42) in addition to soluble APP (Lahiri et al., 1998). The reduction in soluble APP and amyloid-β levels could not be explained by a reduction in APP synthesis and it was suggested that tacrine alters trafficking of APP and/or increased intracellular

proteolysis. In other words, tacrine may reduce the levels of neurotoxic and amyloidogenic amyloid-β-(1–42); also, the neuroprotective soluble APP and the net effect of tacrine needs to be further studied.

3. β-Amyloid peptides as cholinergic neuromodulators

Several lines of evidence suggest a relationship between cholinergic neurotransmission and amyloid-β, and that amyloid-β may act as a physiological active modulator (Auld et al., 1998). Recent studies have shown that low concentrations (picomolar to nanomolar) of amyloid-β can directly induce cholinergic hypofunction. Solubilized amyloid-β inhibits several steps of acetylcholine synthesis and release without inducing any apparent neurotoxicity. Low concentrations of amyloid-β reduced the high-affinity choline uptake and acetylcholine release in slices from rat hippocampus and cortex, but not from striatum (Kar et al., 1996, 1998). Subtoxic levels of amyloid-β disrupted carbachol-induced muscarinic signal transduction in rat cortical neurons and reduced carbachol stimulated GTPase activity (Kelly et al., 1996). In SN 56 cells, derived from mouse basal forebrain cholinergic neurons, amyloid-β-(1–42) and amyloid-β-(1–28) reduced the acetylcholine content of the cells accompanied by proportional decrease in choline acetyltransferase activity (Pedersen et al., 1996). Solubilized amyloid-β-(1–42) suppressed acetylcholine synthesis in rat neuronal cultures and also pyruvate dehydrogenase activity. The major course of reduced acetylcholine synthesis was considered to be an inadequate supply of acetyl–CoA owing to pyruvate dehydrogenase impairment (Hoshi et al., 1997). After in vivo treatment by injecting or infusing amyloid-β into rodent brain, hypofunction has been observed such as reduced choline acetyltransferase markers and levels of acetylcholine (Abe et al., 1994; Giovannelli et al., 1995; Itoh et al., 1996). The findings that amyloid-β disrupts muscarinic receptor coupling to G proteins implicate amyloid-β in the impairment of cholinergic transmission that occurs in Alzheimer's disease. Impaired muscarinic M_1 receptor-G protein coupling would lead to a decreased processing of APP via the α-secretase pathway and potentially increased formation of amyloid-β which in turn might exert additional modulation of the cholinergic system. Recent work by Geula et al. (1998) has shown that aging renders the brain vulnerable to amyloid-β neurotoxicity. Injection of amyloid-β into brains of old rhesus monkeys was neurotoxic whereas it had little toxic effect in young monkeys.

The effect of amyloid-β on nicotinic receptors has so far not been extensively studied. However, recent data imply that amyloid-β as well as hyperphosphorylated tau might suppress gene expression of nicotinic receptors. In cerebral cortex of Alzheimer brain, double-labelling experiments using nicotinic receptor RNA probes and AT8 antibodies against tau demonstrated that neurons heavily labelled for hyperphosphorylated tau seem to express little or no α4 and α7 mRNA. There was no direct correlation between localisation of neurons expressing α4 and α7 mRNA and accumulation of amyloid-β (Wevers et al., 1999). Chronic treatment of PC12 cells with low concentrations of amyloid-β-(25–35) significantly reduced the number of ^{125}I-α-bungarotoxin binding sites, representing α7 nicotinic receptor subtype, and the level of α7 subunit mRNA (X. Zhang, personal communication).

4. Neurotransmitter regulation of tau protein

Another hallmark of Alzheimer's disease is the neurofibrillary lesions consisting of neurofibrillary tangles, neuropil threads, and dystrophic neurites. The principal structural component of neurofibrillary lesions are paired helical filaments, which are made up of microtubule-associated protein tau in a hyperphosphorylated state (for reviews, see Spillantini and Goedert, 1997; Johnson and Hartigan, 1998). Hyperphosphorylation of tau has been shown to dissociate tau from microtubules, leading to disruption of the neuronal cytoskeleton and interference with intracellular transport mechanisms (Vincent et al., 1994).

An imbalance of protein kinase/protein phosphatase system, either as an increased activity of protein kinase, and/or decreased activity of protein phosphatase may contribute to the hyperphosphorylation of tau in Alzheimer's disease. Many kinases have been shown to phosphorylate tau in vitro, including Ca^{2+}/calmodulin dependent kinase II, mitogen-activated protein kinases and glycogen synthetase kinase-3β (Billingsley and Kincaid, 1997; Johnson and Hartigan, 1998). However, the mechanism that regulates the activities of brain protein kinases and phosphatases and thus determine the pattern and extent of tau phosphorylation in vivo are not fully understood. In general, the number of neurofibrillary tangles correlates more closely with the severity of dementia and loss of synapses and neurons (Gomez-Isla et al., 1997; Terry, 1996) than the number of plaques. A highly correlative relationship between cortical distribution of hyperphosphorylated tau in Alzheimer brain and cholinergic depletion early during the course of the disease was demonstrated by Arendt et al. (1999). Compared to studies investigating modulation of APP processing by stimulating cholinergic receptors, few works have focused on the role of these receptors in regulation of tau protein phosphorylation.

4.1. Muscarinic receptors

A stimulation of PC12 cells transfected with the gene for muscarinic M_1 receptor with the agonists carbachol and AF 102B has been reported to decrease the levels of phosphorylated tau (AT8 and PHF1 immunoreactivity). This effect was both time-and dose-dependent, and was

blocked by atropine (Sadot et al., 1996). Recently, it was shown that tau of apolipoprotein E-deficient mice contains a domain of hyperphosphorylated epitopes and that treatment of these mice with the muscarinic M_1 receptor agonist AF150(S) can reduce this excess phosphorylation of tau (Genis et al., 1999). Furthermore, treatment of these mice with AF150(S) abolished their memory deficits and resulted in recovery of their brain cholinergic markers (Fisher et al.,1998). The inverse relationship between muscarinic receptor activation and tau phosphorylation suggests a link between the cholinergic signal transduction system and the neuronal cytoskeleton. The decreased cholinergic activity associated with Alzheimer's disease may contribute to an increased phosphorylation of tau and destabilization of the microtubule and cell death.

4.2. Nicotinic receptors

This is an area of research where few data are available despite the fact that a loss of nicotinic receptors is consistently found in brain of Alzheimer patients. Using SH-SY5Y human neuroblastoma cells, we found that treatment with nicotine and epibatidine increased the levels of phosphorylated tau (AT8 and AT 270 immunreactivity) as well as nonphosphorylated tau (Tau-1 immunoreactivity) (Hellström-Lindahl et al., 2000). The antagonists D-tubocurarine and mecamylamine prevented the increase in tau immunoreactivity, but failed to block the effect of nicotine or epibatidine on up-regulation of nicotinic receptors. Thus, activation of nicotinic receptors seems to be required for affecting intracellular levels of tau but not for the up-regulation of the receptors induced by nicotine or epibatidine in these cells. The effect of cholinesterase inhibitors on tau phosphorylation was also investigated since as aforementioned, several cholinesterase inhibitors have been shown to interact with the nicotinic receptors. Similar to nicotine, treatment with the cholinesterase inhibitors tacrine, donepezil and galanthamine all increased levels of phosphorylated tau and up-regulated the number of nicotinic receptors. The increased tau immunoreactivity induced by tacrine was prevented by nicotinic antagonists but not by the muscarinic receptor antagonist atropine, indicating that in terms of acetylcholine receptors, tacrine modulates tau levels mainly through interactions with nicotinic receptors and not with muscarinic receptors. Whether the increase in tau phosphorylation also leads to abnormal tau aggregation and contributes to formation of paired helical filaments remains to be studied.

The exact molecular mechanism by which nicotinic receptor activation induces changes in tau levels and phosphorylation remains unclear. It is also unknown if stimulation of other nicotinic receptor subtypes, like $\alpha 4\beta 2$, produces similar effects as those seen in SH-SY5Y cells which express the $\alpha 3$ and $\alpha 7$ subtype. Nicotinic receptors are permeable for Ca^{2+} and its entry through the receptor is sufficient to activate various Ca^{2+} dependent cellular processes. The nicotine-induced increase in phosphorylated tau was calcium-dependent since removal of Ca^{2+} by addition of EGTA into culture medium prevented this increase. A similar effect of EGTA have been shown for the glutamate-induced increase in tau phosphorylation (Mattson, 1990). Moreover, treatment with the Ca^{2+} ionophore A23187 in the presence of extracellular Ca^{2+} induced an increased phosphorylation of tau (Mattson,1990; Shea et al., 1996). This increase was prevented by inhibitors against calpain or the Ca^{2+} dependent protein kinase C, whereas activation of protein kinase C increased immunoreactivity, suggesting that these enzymes were required and perhaps involved one or more kinase cascades to mediate the effect of Ca^{2+} influx on tau immunoreactivity (Shea et al., 1996).

5. Tau protein and amyloid-β interactions

A central issue in the pathogenesis of Alzheimer's disease is the relationship between amyloid deposition and neurofibrillary tangle formation. Several lines of evidence suggest that increased phosphorylation of tau by amyloid-β could contribute to the amyloid-β neurotoxicity. In Alzheimer brain, nanomolar concentration of tau binds to senile plaque in situ in cortical and subcortical regions by a direct interaction with a transmembrane region of APP 714–723, which is a component of senile plaques (Smith et al., 1995). Several studies have shown that treatment of different cell cultures with amyloid-β can induce tau phosphorylation (Busciglio et al., 1995; Takashima et al., 1996; Le et al., 1997; Shea et al., 1997). In vivo, Sigurdsson et al. (1995) demonstrated the amyloid-β-(25–35) induced immunoreactivity of phosphorylated tau following injections into amygdala of rats. Amyloid-β-(25–35) also evoked tau phosphorylation in SH-SY5Y neuroblastoma cells (Shea et al., 1997). In primary cultures of fetal rat hippocampus and human cortical neurons, fibrillar amyloid-β, but not soluble or aggregated amyloid-β, induced the phosphorylation of tau, resulting in loss of microtubule binding capacity (Busciglio et al., 1995). Using SN 56 cells, Le et al. (1997) showed that treatment with aggregated amyloid-β-(1–40) caused cell injury, increased levels of phosphorylated tau as well as total tau. Interestingly, amyloid-β-(1–40) also increased the levels of cell-associated and secreted APP; furthermore, secreted forms of APP can enhance phosphorylation of tau (Greenberg et al., 1994). Thus, amyloid-β can increase expression of APP, and tau phosphorylation induced by APP may contribute to amyloid-β neurotoxicity as opposed to the neuroprotective effects of APP. The mechanisms by which amyloid-β induce phosphorylated tau are not clear but can be a result of increased activities of protein kinases (Greenberg et al., 1994; Takashima et al., 1996). These studies suggest a potential link between amyloid-β aggregation, cytoskeletal abnormalities and cell death, and that neurofibrillary tan-

gles and senile plaques may play a role in each other's formation during pathogenesis of Alzheimer's disease.

6. Neuroprotective effect of nicotine and smoking

In earlier epidemiological studies, smoking has been associated with a reduced risk of Alzheimer's disease (Lee, 1994). The number of nicotinic receptors are increased in brains of smokers (Benwell et al., 1988; Court et al., 1998, Perry et al., 1999) and the neuroprotective effects may be mediated by the stimulation of nicotinic receptors. However, two recent prospective population-based studies on smoking and risk of Alzheimer's disease showed in comparison with those who never smoked that smokers had a twofold increased risk of dementia and Alzheimer's disease (Ott et al., 1998; Merchant et al., 1999). Among previous smokers who quit smoking, there was an indication of slight reduction in the risk of Alzheimer's disease (Merchant et al., 1999). Although the findings from epidemiological studies are somewhat contradicting, in vitro data suggest a neuroprotective effect of nicotine and nicotinic agonists. Incubating rat cortical neurons with nicotine significantly reduces amyloid-β-(25–35) cytotoxicity (Kihara et al., 1998). This neuroprotective effect was blocked by dihydro-beta-erytroidine and $\alpha 4\beta 2$ nicotinic receptor antagonist. Nicotine was also found to attenuate the neurotoxicity of amyloid-β-(25–35) in rat hippocampal neuronal cultures, an effect which was significantly inhibited by the nicotinic receptor antagonist mecamylamine (Zamani et al., 1997). Furthermore, the cholinesterase inhibitors tacrine and donepezil were found in clinical relevant concentrations to attenuate amyloid-β-(25–35) induced toxicity in PC 12 cells (Svensson and Nordberg, 1998). The effect of tacrine was blocked by mecamylamine and D-tubocurarine, suggesting an interaction with nicotinic receptors. Nicotine has also been shown, via stimulation of $\alpha 7$ nicotinic receptors, to protect against neurotoxicity induced by glutamate (Akaike et al., 1994; Shimohama et al., 1998) and nerve growth factor deprivation (Li et al.,1999). The mechanism by which nicotine is neuroprotective is not clear but might involve the desensitization and/or up-regulation of nicotinic receptors induced by chronic nicotine exposure, increased release of neurotrophic soluble APP by nicotine, or involve regulation of nitric oxide synthase.

A few studies have investigated whether smoking exerts any influence on a number of senile plaques and neurofibrillary tangle formation. Ulrich et al. (1997) found significantly reduced senile plaques in female smokers compared to non-smokers. This is in agreement with a study by Court et al. (1998) demonstrating that mean density of neocortical plaques tended to be higher in non-smokers than in smokers, whereas ex-smoking values were intermediate. The relationship between nicotinic receptors and β-amyloidosis have been investigated in a large series of non-demented and demented individuals, and demonstrated a greater age-related loss of nicotine binding in areas earliest affected by plaques (Perry et al., 2000). There was a significant correlation between amyloid-β-(1–42) and density of nicotinic receptors whereas formation of neurofibrillary tangles was not correlated to a number of nicotinic receptors. In vitro studies have shown that nicotine may inhibit deposition of amyloid-β by stabilizing the α-helical structure of the β-peptide and thereby prevent β-sheet formation (Salomon et al., 1996). This is in contrast to the findings reported by Kihara et al. (1999) demonstrating that nicotinic receptor agonists did not influence the formation of the β-sheet structure.

7. Concluding remarks

Aberrant processing of APP is central to the formation of amyloid-β and senile plaque present in Alzheimer brain. It is not clear which is the first step in the generation of Alzheimer's disease but it seems that the cholinergic system, amyloid-β production and formation of neurofibrillary tangles are closely related. It is possible that APP mismetabolism as a result of impaired cholinergic neurotransmission may contribute to amyloidogenesis. Neurofibrillary tangles and senile plaques, often thought as independent structures, may play a role in each other's formation during pathogenesis of Alzheimer's disease. Many factors have been shown to influence the processing of APP and phosphorylation of tau, including activation of actylcholine receptors. Considerable in vitro evidence exists for the neuroprotective effects of nicotine and related agents. Compared to effects demonstrated by activation of nicotinic and muscarinic receptors in cell models, regulation of APP processing and tau phosphorylation in the intact brain may be much more complex in that different receptors may interact simultaneously. It is also clear that, in brain, there are several additional factors that may contribute to the formation of paired helical filaments or the aggregation of amyloid-β and thereby induce neurodegeneration.

Acknowledgements

This work was financially supported by grants from the Swedish Medical Research Council (project no. 05817), Stiftelsen för Gamla Tjänarinnor, Åke Wiberg's foundation, Loo and Hans Osterman's foundation, Alzheimer Foundation Sweden, Stohne's foundation, M. Bergwall Foundation, KI foundations and the Council for Medical Tobacco Research, Swedish Match.

References

Abe, E., Casamenti, F., Giovannelli, L., Scali, C., Pepeu, G., 1994. Administration of amyloid beta-peptides into the medial septum of rats decreases acetylcholine release from hippocampus in vivo. Brain Res. 636, 162–164.

Akaike, A., Tamura, Y., Yokota, T., Shimohama, S., Kimura, J., 1994. Nicotine-induced protection of cultured cortical neurons against *N*-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. Brain Res. 644, 181–187.

Arendt, T., Holzer, M., Gertz, H.-J., Brückner, M.K., 1999. Cortical load of PHF-tau in Alzheimer's disease is correlated to cholinergic dysfunction. J. Neural. Trans. 106, 513–523.

Auld, D.S., Kar, S., Quirion, R., 1998. β-Amyloid peptides as direct cholinergic neuromodulators: a missing link? Trends Neurosci. 21, 43–49.

Benwell, M., Balfour, D., Andersson, J., 1988. Evidence that tobacco smoking increases the density of (-)[^{3}H] nicotine binding sites in human brain. J. Neurochem. 50, 1242–1247.

Billingsley, M.L., Kincaid, R.L., 1997. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. Biochem. J. 323, 577–591.

Busciglio, J., Lorenzo, A., Yeh, J., Yankner, B., 1995. β-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. Neuron 14, 879–888.

Buxbaum, J.D., Oishi, M., Chen, H.I., Pinkas-Kramarski, R., Jaffe, E.A., Gandy, S.E., Greengard, P., 1992. Cholinergic agonists and interleukin-1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. Proc. Natl. Acad. Sci. USA 89, 10075–10078.

Buxbaum, J.D., Koo, E.H., Greengard, P., 1993. Protein phosphorylation inhibits production of Alzheimer amyloid β/A4 peptide. Proc. Natl. Acad. Sci. USA 90, 9195–9198.

Buxbaum, J.D., Ruefli, A.A., Parker, C.A., Cypress, A.A., Greengard, P., 1994. Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. Proc. Natl. Acad. Sci. USA 91, 4489–4493.

Checler, F., 1995. Processing of the β-amyloid precursor protein and its regulation in Alzheimer's disease. J. Neurochem. 65, 1431–1444.

Cook, D.G., Forman, M.S., Sung, J.C., Leight, S., Kolson, D.L., Iwatsubo, T., Lee, V.M.L., Doms, R.W., 1997. Alzheimer's Aβ (1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nat. Med. 3, 1021–1023.

Court, J.A., Lloyd, S., Thomas, N., Piggott, M.A., Marshall, E.F., Morris, C.M., Lamb, H., Perry, R.H., Johnson, M., Perry, E.K., 1998. Dopamine and nicotinic receptor binding and the levels of dopamine and homovanillic acid in human brain related to tobacco use. Neuroscience 87, 63–78.

Dyrks, T., Mönning, U., Beyreuther, K., Turner, J., 1994. Amyloid precursor protein secretion and βA4 amyloid generation are not mutually exclusive. FEBS Lett. 349, 210–214.

Efthimiopoulos, S., Vassilacopoulou, D., Ripellino, J., Tezapsidis, N., Robakis, N.K., 1996. Cholinergic agonists stimulate secretion of soluble full-length amyloid precursor protein in neuroendocrine cells. Proc. Natl. Acad. Sci. USA 93, 8046–8050.

Fisher, A., Brandeis, R., Chapman, S., Pittel, Z., Michaelson, D.M., 1998. M1 muscarinic agonist treatment reverses cognitive and cholinergic impairment of apolipoprotein E-deficient mice. J. Neurochem. 70, 1991–1997.

Gandy, S., Greengard, P., 1994. Regulated cleavage of the Alzheimer amyloid precursor protein: molecular and cellular basis. Biochimie 76, 300–303.

Genis, I., Fisher, A., Michaelson, D.M., 1999. Site-specific dephosphorylation of tau of apolipoprotein E-deficient and control mice by M_1 muscarinic agonist treatment. J. Neurochem. 72, 206–213.

Geula, C., Wu, C.K., Saroff, D., Lorenzo, A., Yuan, M., Yankner, B.A., 1998. Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. Nat. Med. 4, 827–831.

Giovannelli, L., Casamenti, F., Scali, L., Bartolini, L., Pepeu, G., 1995. Differential effects of amyloid peptides β-(1–40) and β-(25–35) injections into the rat nucleus basalis. Neuroscience 66, 781–792.

Gomez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J.H., Petersen, R.C., Parisi, J.E., Hyman, B.T., 1997. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann. Neurol. 41, 17–24.

Greenberg, S.M., Koo, E.H., Selkoe, D.J., Qui, W.Q., Kosik, K.S., 1994. Secreted β-amyloid precursor protein stimulates mitogen-activated protein kinase and enhances tau phosphorylation. Proc. Natl. Acad. Sci. USA 91, 7104–7108.

Haass, C., Schlossmacher, M.G., Hung, A.Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B.L., Lieberburg, I., Koo, E.H., Schenk, D., Teplow, D.B., Selkoe, D.J., 1992. Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 359, 322–325.

Hardy, J., 1997. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci. 20, 154–159.

Hartmann, T., Bieger, S.C., Bruhl, B., Tienari, P.J., Ida, N., Allsop, D., Roberts, G.W., Masters, C.L., Dotti, C.G., Unsicker, K., Beyreuther, K., 1997. Distinct sites of intracellular production for Alzheimer's disease Aβ40/42 amyloid peptides. Nat. Med. 3, 1016–1020.

Hellström-Lindahl, E., Moore, H., Nordberg, A., 2000. Increased levels of tau protein in SH-SY5Y cells after treatment with cholinesterase inhibitors and nicotinic agonists. J. Neurochem. 74, in press.

Holladay, M., Dart, M., Lynch, J.K., 1997. Neuronal nicotinic acetylcholine receptors as targets for drug discovery. J. Med. Chem. 40, 4169–4194.

Hoshi, M., Takashima, A., Murayama, M., Yasutake, K., Yoshida, N., Ishiguro, K., Hoshino, T., Imahori, K., 1997. Nontoxic amyloid β peptide$_{1-42}$ suppresses acetylcholine synthesis. J. Biol. Chem. 272, 2038–2041.

Hung, A.Y., Haass, C., Nitsch, R.M., Qui, W.Q., Citron, M., Wurtman, R.J., Growdon, J.H., Selkoe, D., 1993. Activation of protein kinase C inhibits cellular production of the amyloid β-protein. J. Biol. Chem. 268, 22959–22962.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal system in beta-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Johnson, G.V.W., Hartigan, J.A., 1998. Tau protein in normal and Alzheimer's disease brain: an update. Alzheimer's Dis. Rev. 3, 125–141.

Kar, S., Seto, D., Gaudreau, P., Quirion, R., 1996. β-amyloid-related peptides inhibit potassium-evoked acetylcholine release from rat hippocampal slices. J. Neurosci. 16, 1034–1040.

Kar, S., Issa, M., Seto, D., Auld, D.S., Collier, B., Quirion, R., 1998. Amyloid β-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. J. Neurochem. 70, 2179–2187.

Kelly, J.F., Furukawa, K., Barger, S.W., Rengen, M.R., Mark, R.J., Blanc, E.M., Roth, G.S., Mattson, M.P., 1996. Amyloid β-peptide disrupts carbachol-induced muscarinic cholinergic signal transduction in cortical neurons. Proc. Natl. Acad. Sci. USA 93, 6753–6758.

Kihara, T., Shimohama, S., Urushitani, M., Sawada, H., Kimura, J., Kume, T., Maeda, T., Akaike, A., 1998. Stimulation of α4β2 nicotinic acetylcholine receptors inhibits β-amyloid toxicity. Brain Res. 792, 331–334.

Kihara, T., Shimohama, S., Akaike, A., 1999. Effects of nicotinic receptor agonists on beta-amyloid beta-sheet formation. Jpn. J. Pharmacol. 79, 393–396.

Kim, S.-H., Kim, Y.-K., Jeong, S.-J., Haas, C., Kim, Y.-H., Suh, Y.-H., 1997. Enhanced release of secreted form of Alzheimer's amyloid precursor protein from PC12 cells by nicotine. Mol. Pharmacol. 52, 430–436.

Kuisak, J.W., Northon, D.D., Sisodia, S.S., Zhao, B., 1999. The regula-

tion of amyloid precursor protein processing by nicotinic cholinergic mechanisms. Soc. Neurosci. Abstr. 25, 598.

Ladner, C.J., Lee, J.M., 1998. Pharmacological drug treatment of Alzheimer disease: the cholinergic hypothes is revisited. J. Neuropathol. Exp. Neurol. 57, 719–731.

Lahiri, D.K., Lewis, S., Farlow, M., 1994. Tacrine alters the secretion of the beta-amyloid precursor protein in cell lines. J. Neurosci. 37, 777–787.

Lahiri, D.K., Farlow, M., Sambamurti, K., 1998. The secretion of amyloid β-peptides is inhibited in the tacrine-treated human neuroblastoma cells. Mol. Brain Res. 62, 131–140.

Le, W., Xie, W.J., Kong, R., Appel, S., 1997. β-amyloid-induced neurotoxicity of a hybrid septal cell line associated with increased tau phosphorylation and expression of β-amyloid precursor protein. J. Neurochem. 69, 978–985.

Lee, P.N., 1994. Smoking and Alzheimer's disease: a review of the epidemiological evidence. Neuroepidemiology 13, 13–144.

Li, Y., Papke, R.L., He, Y.-J., Millard, W.J., Meyer, E.M., 1999. Characterization of the neuroprotective and toxic effects of α7 nicotinic receptor activation in PC12 cells. Brain Res. 830, 218–225.

Loeffler, J., Huber, G., 1993. Modulation of β-amyloid precursor protein secretion in differentiated and nondifferentiated cells. Biochem. Biophys. Res. Commun. 195, 97–103.

Maelicke, A., Schrattenholz, A., Storch, A., Schröder, B., Gutbrod, O., Methfessel, C., Weber, K.-H., Pereira, E., Alkondon, M., Albuquerque, E.X., 1995. Noncompetitive agonism at nicotinic acetylcholine receptors; functional significance for CNS signal transduction. J. Recept. Signal Transduction Res. 15, 333–353.

Mattson, M.P., 1990. Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and Ca^{2+} influx in cultured hippocampal neurons. Neuron 2, 105–117.

Mattson, M.P., 1997. Cellular aspects of β-amyloid precursor protein and its soluble and fibrillogenic derivatives. Physiol. Rev. 77, 1081–1132.

Merchant, C., Tang, M.-X., Albert, S., Manly, J., Stern, Y., Mayeux, R., 1999. The influence of smoking on the risk of Alzheimer's disease. Neurology 52, 1408–1412.

Mills, J., Reiner, P.B., 1999. Regulation of amyloid precursor protein cleavage. J. Neurochem. 72, 443–460.

Mori, F., Lai, C.-C., Fusi, F., Giacobini, E., 1995. Cholinesterase inhibitors increase secretion of APPs in rat brain cortex. NeuroReport 6, 633–636.

Newhouse, P., Potter, A., Levin, E., 1997. Nicotinic system involvement in Alzheimer's and Parkinson's diseases, implications for therapeutics. Drugs Aging 11, 206–228.

Nitsch, R.M., Growdon, J.H., 1994. Role of neurotransmission in the regulation of amyloid β-protein precursor processing. Biochem. Pharmacol. 47, 1275–1284.

Nitsch, R.M., Slack, B.E., Wurtman, R.J., Growdon, J.H., 1992. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptor. Science 258, 304–307.

Nitsch, R.M., Farber, S.A., Growdon, J.H., Wurtman, R., 1993. Release of amyloid β-protein precursor derivatives by electrical depolarization of rat hippocampal slices. Proc. Natl. Acad. Sci. USA 90, 5191–5193.

Nitsch, R.M., Deng, M., Growdon, J.H., Wurtman, R., 1996. Serotonin 5-HT2a and 5-HT2c receptors stimulate amyloid precursor protein ectodomain secretion. J. Biol. Chem. 271, 4188–4194.

Nitsch, R.M., Kim, C., Growdon, J.H., 1998. Vasopressin and bradykinin regulate secretory processing of the amyloid protein precursor of Alzheimer's disease. Neurochem. Res. 23, 807–814.

Nordberg, A., 1992. Neuroreceptor changes in Alzheimer disease. Cerebrovasc. Brain Metab. Rev. 4, 303–328.

Nordberg, A., Svensson, A.-L., 1998. Cholinesterase inhibitors in the treatment of Alzheimer's disease. A comparison of tolerability and pharmacology. Drug Safety 19, 465–480.

Nordberg, A., Lilja, A., Lundqvist, H., Hartwig, P., Amberla, K., Viitanen, M., Warpman, U., Johansson, M., Hellström-Lindahl, E., Bjurling, P., Fasth, K.-J., Långström, B., Winblad, B., 1992. Tacrine restores cholinergic nicotinic receptors and glucose metabolism in Alzheimer patients as visualized by positron emission tomography. Neurobiol. Aging 13, 747–758.

Ott, A., Slooter, A.J.C., Hofman, A., van Harskamp, F., Witteman, J.C.M., Van Broeckhoven, C., van Dijn, C.M., 1998. Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam study. Lancet 351, 1840–1843.

Pavia, J., de Ceballos, M.L., Sanchez de la Cuesta, F., 1998. Alzheimer's disease: relationship between muscarinic cholinergic receptors, β-amyloid and tau proteins. Fundam. Clin. Pharmacol. 12, 473–481.

Pedersen, W.A., Kloczewiak, M.A., Blusztajn, J.K., 1996. Amyloid β-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. Proc. Natl. Acad. Sci. USA 93, 8068–8071.

Pereira, E.F.R., Alkondon, M., Reinhardt, S., Maelicke, A., Peng, X., Lindstrom, J., Whiting, J., Albuquerque, E.X., 1994. Physostigmine and galanthamine characterize the presence of a novel binding site on the α4β2 subtype of neuronal nicotinic acetylcholine receptors stably expressed in fibroblast cells. J. Pharmacol. Exp. Ther. 270, 768–778.

Perry, D.C., Davila-Garcia, M.I., Stockmeier, C.A., Kellar, K., 1999. Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. J. Pharmacol. Exp. Ther. 289, 1545–1552.

Perry, E.K., Martin Ruiz, C., Lee, M., Griffiths, M., Johnson, M., Piggott, M., Haroutunia, H., Davis, K., Gotti, C., Tzartos, R., Cohen, O., Soreq, H., Jaros, E., Perry, R., Ballard, C., McKeith, I., Court, J., 2000. Nicotinic receptor subtypes in human brain aging, Alzheimer and Levy body diseases. Eur. J. Pharmacol. 1, (this Proceedings).

Pittel, Z., Heldman, E., Barg, J., Haring, R., Fisher, A., 1996. Muscarinic control of amyloid precursor protein secretion in rat cerebral cortex and cerebellum. Brain Res. 742, 299–304.

Querfurth, H.W., Selkoe, D.J., 1994. Calcium ionophore increases amyloid β-peptide production by cultured cells. Biochemistry 33, 4550–4561.

Sabbagh, M., Galsko, D., Thal, J., 1997. β-amyloid and treatment opportunities for Alzheimer's disease. Alzheimer's Dis. Rev. 3, 1–19.

Sadot, E., Gurwitz, D., Barg, J., Behar, L., Ginzburg, I., Fisher, A., 1996. Activation of m1 muscarinic acetylcholine receptor regulates τ phosphorylation in transfected PC12 cells. J. Neurochem. 66, 877–880.

Salomon, A.R., Marcinowski, K.J., Friedland, R.P., Zagorski, M.G., 1996. Nicotine inhibits amyloid formation by the β-peptide. Biochemistry 35, 13568–13578.

Selkoe, D.J., 1996. Amyloid β-protein and the genetics of Alzheimer's disease. J. Biol. Chem. 271, 18295–18298.

Selkoe, D.J., 1999. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 399, 23–31.

Shea, T.B., Spencer, M.J., Beerman, M.L., Cressman, C.M., Nixon, R.A., 1996. Calcium influx into human neuroblastoma cells induces ALZ-50 immunoreactivity: involvement of calpain-mediated hydrolysis of protein kinase C. J. Neurochem. 66, 1539–1549.

Shea, T.B., Prabhakar, S., Ekinci, F., 1997. β-amyloid and ionophore A23187 evoke tau hyperphosphorylation by distinct intracellular pathways:differential involvement of the calpain/protein kinase C system. J. Neurosci. Res. 49, 759–768.

Shimohama, S., Greenwald, D.L., Shafron, D.H., Akaika, A., Maeda, T., Kaneko, S., Kimura, J., Simpkins, C.E., Day, A.L., Meyer, E.M., 1998. Nicotinic α7 receptors protect against glutamate neurotoxicity and neuronal ischemic damage. Brain Res. 779, 359–363.

Sigurdsson, E.M., Hejna, M.J., Magnusson, D.J., Lee, J.M., Lorens, S.A., 1995. β-amyloid 25–35 (βA) induced tau-immunoreactivity following injections into the amygdala of rats in vivo. Soc. Neurosci. Abstr. 21, 1719.

Sindou, P., Lesort, M., Couratier, P., Yardin, C., Esclaire, F., Hugon, J., 1994. Glutamate increases tau phosphorylation in primary neuronal cultures from fetal rat cerebral cortex. Brain Res. 646, 124–128.

Slack, B.E., Breu, J., Petryniak, M.A., Srivastava, K., Wurtman, R.J., 1995. Tyrosine phosphorylation-dependent stimulation of amyloid

precursor protein secretion by the m3 muscarinic acetylcholine receptor. J. Biol. Chem. 270, 8337–8344.

Small, D.H., McLean, C.A., 1999. Alzheimer's disease and the amyloid β protein: what is the role of amyloid? J. Neurochem. 73, 443–449.

Smith, M.A., Siedlak, S.L., Richey, P.L., Mulvihill, P., Ghiso, J., Frangione, B., Tagliavini, F., Giaccone, G., Bugiani, O., Praprotnik, D., Kalaria, R.N., Perry, G., 1995. Tau protein directly interacts with the amyloid β-protein precursor: implication for Alzheimer's disease. Nat. Med. 1, 365–370.

Spillantini, M.G., Goedert, M., 1997. Tau protein pathology in neurodegenerative diseases. Trends Neurosci. 21, 428–433.

Svensson, A.-L., Nordberg, A., 1996. Tacrine interacts with an allosteric activator site on α4β2 nAChRs in M10 cells. NeuroReport 7, 2201–2205.

Svensson, A.-L., Nordberg, A., 1997. Interaction of tacrine, galanthamine, NXX-066 and E2020 with neuronal α4β2 nicotinic receptors expressed in fibroblast cells. In: Iqbal, K., Winblad, B., Nishimura, T., Taked, M., Wisniewski, H.M. (Eds.), Alzheimer's Disease: Biology, Diagnosis and Therapeutics. Wiley, pp. 753–758.

Svensson, A.L., Nordberg, A., 1998. Tacrine and donepezil attenuate the neurotoxic effect of $A\beta_{(25-35)}$ in rat PC12 cells. NeuroReport 9, 1519–1522.

Takashima, A., Nogushi, K., Michel, G., Hoshi, M., Ishiguro, K., Imahori, K., 1996. Exposure of rat hippocampal neurons to amyloid β-peptide (25–35) induces the inactivation of phosphatidyl inositol-3 kinase and the activation of tau protein kinase I/glycogen synthase kinase-3β. Neurosci. Lett. 203, 33–36.

Terry, R.D., 1996. The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. J. Neuropathol. Exp. Neurol. 55, 1023–1025.

Ulrich, J., Johannson-Locher, G., Seiler, W.O., Stähelin, H.B., 1997. Does smoking protect from Alzheimer's disease? Alzheimer-type changes in 301 unselected brains from patients with known smoking history. Acta Neuropathol. 94, 450–454.

Vincent, I., Rosado, M., Kim, E., Davies, P., 1994. Increased production of paired helical filament epitopes in a cell culture system reduces the turnover of τ. J. Neurochem. 62, 715–723.

Wevers, A., Monteggia, L., Nowacki, S., Bloch, W., Schütz, U., Linstrom, J., Pereira, E.F.R., Eisenberg, H., Giacobini, E., de Vos, R.A.I., Jansen Steur, E.N.H., Maelicke, A., Albuquerque, E.X., Schröder, H., 1999. Expression of nicotinic acetylcholine receptor subunits in the cerebral cortex in Alzheimer's disease: histotopographical correlation with amyloid plaques and hyperphosphorylated-tau protein. Eur. J. Neurosci. 11, 2551–2565.

Wolf, B.A., Wertkin, A.M., Jolly, Y.C., Yasuda, R.P., Wolfe, B.B., Konrad, R.J., Manning, D., Ravi, S., Williamson, J.R., Lee, V.M.-Y., 1995. Muscarinic regulation of Alzheimer's disease amyloid precursor protein secretion and amyloid β-protein production in human NT2N cells. J. Biol. Chem. 270, 4916–4922.

Zamani, M.R., Allen, Y.S., Owen, G.P., Gray, J.A., 1997. Nicotine modulates the neurotoxic effect of β-amyloid $protein_{(25-35)}$ in hippocampal cultures. NeuroReport 8, 513–517.