Review

The cholinergic system in aging and neuronal degeneration

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A B S T R A C T

The basal forebrain cholinergic complex comprising medial septum, horizontal and vertical diagonal band of Broca, and nucleus basalis of Meynert provides the mayor cholinergic projections to the cerebral cortex and hippocampus. The cholinergic neurons of this complex have been assumed to undergo moderate degenerative changes during aging, resulting in cholinergic hypofunction that has been related to the progressing memory deficits with aging.

However, the previous view of significant cholinergic cell loss during aging has been challenged. Neuronal cell loss was found predominantly in pathological aging, such as Alzheimer’s disease, while normal aging is accompanied by a gradual loss of cholinergic function caused by dendritic, synaptic, and axonal degeneration as well as a decrease in trophic support. As a consequence, decrements in gene expression, impairments in intracellular signaling, and cytoskeletal transport may mediate cholinergic cell atrophy finally leading to the known age-related functional decline in the brain including aging-associated cognitive impairments.

However, in pathological situations associated with cognitive deficits, such as Parkinson’s disease, Down-syndrome, progressive supranuclear palsy, Jakob–Creutzfeld disease, Korsakoff’s syndrome, traumatic brain injury, significant degenerations of basal forebrain cholinergic cells have been observed. In presenile (early onset), and in the advanced stages of late-onset Alzheimer’s disease (AD), a severe loss of cortical cholinergic innervation has extensively been documented. In contrast, in patients with mild cognitive impairment (MCI, a prodromal stage of AD), and early forms of AD, apparently no cholinergic neurodegeneration but a loss of cholinergic function occurs. In particular imbalances in the expression of NGF, its precursor proNGF, the high and low NGF receptors, trkA and p75NTR, respectively, changes in acetylcholine release, high-affinity choline uptake, as well as alterations in muscarinic and nicotinic acetylcholine receptor expression may contribute to the cholinergic dysfunction. These observations support the suggestion of a key role of the cholinergic system in the functional processes that lead to AD. Malfunction of the cholinergic system may be tackled pharmacologically by intervening in cholinergic as well as neurotrophic signaling cascades that have been shown to ameliorate the cholinergic deficit at early stages of the disease, and slow-down the progression. However, in contrast to many other, dementing disorders, in AD the cholinergic dysfunctions are accompanied by the occurrence of two major histopathological hallmarks such as β-amyloid plaques and neurofibrillary tangles, provoking the question whether they play a particular role in inducing or mediating cholinergic dysfunction in AD. Indeed, there is abundant evidence that β-amyloid may trigger cholinergic dysfunction through action on α7 nicotinic acetylcholine receptors, affecting NGF signaling, mediating tau phosphorylation, interacting with acetylcholinesterase, and specifically affecting the proteome in cholinergic neurons. Therefore, an early onset of an anti β-amyloid strategy may additionally be potential in preventing aging-associated cholinergic deficits and cognitive impairments.

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1. Basal forebrain cholinergic system in postnatal development and aging

Acetylcholine is widely distributed in the nervous system and has been implicated to play a critical role in cerebral cortical development, cortical activity, controlling of cerebral blood flow, and sleep–wake cycle as well as in modulating cognitive performances and learning and memory processes (for references, see e.g. [1]). It plays an essential role in structural and functional remodeling of cortical circuits by establishing synaptic contacts in networks of cells that will subserve complex cognitive functions in adulthood [2]. The basal forebrain cholinergic complex comprising medial septal, horizontal and vertical diagonal band of Broca, and nucleus basalis of Meynert provides the major cholinergic projections to the cerebral cortex and hippocampus. The cholinergic neurons of this complex have been described to undergo moderate degenerative changes during aging, resulting in cholinergic hypofunction that has been related to the progressing memory deficits with aging [1,3,4]. The preferential age-dependent degeneration of basal forebrain cholinergic neurons might be due to a decrease in trophic support, as in aging an attenuation of neurotrophic factors might be due to a decrease in trophic support, as in aging an attenuation of neurotrophic factors from lesion-induced atrophy [8–10].

However, the aging-related cholinergic atrophy and cell loss in normal brain is not complemented by reductions in the levels of NGF [11], which hints to a functional loss of aging cholinergic neurons to respond to NGF. Indeed, in aged rats, forebrain cholinergic neurons demonstrated striking reductions in the retrograde transport of NGF, and cholinergic cells that were no longer capable to transport NGF appeared severely shrunken [12,13]. Thus basal forebrain cholinergic neurons seem to be particularly sensitive to failures in NGF signaling that may induce cellular atrophy and changes in gene expression [14].

Aging-related atrophy of basal forebrain cholinergic neurons has also been observed in the non-human primate brain. Short-term reversal of this atrophy has been reported following intracerebroventricular NGF infusion [15,16] or ex vivo NGF gene delivery [17–20]. Sustained intracerebroventricular NGF administration has been reported to induce adverse side effects [21], whereas a clinical trial of NGF gene therapy in individuals with mild Alzheimer disease (AD) for 22 months did not display long-term adverse effects of NGF, but delayed the rate of cognitive decline [22]. This is supported by a recent study reporting that long-term intra-parenchymal lentiviral NGF gene delivery to the cholinergic basal forebrain of aged rhesus monkeys significantly restored cholinergic neuronal markers to levels of young monkeys, indicating that extended trophic support to neurons may reverse aging-related neuronal atrophy [23]. However, the previous view of wide neuronal cell loss during aging has been challenged, partly due to improved stereological techniques [24]. Neuronal cell loss was found predominantly in pathological aging, such as AD, while normal aging is accompanied by dendritic, synaptic, and axonal degeneration with nearly no cell loss [25–30]. These findings suggest that functional decline associated with aging across species does not primarily result from cell loss, but other mechanisms including decrements in gene expression, impairments in intracellular signaling, and cytoskeletal transport that may mediate cholinergic cell atrophy leading to age-related functional decline in the brain [13,31–33].

The aging brain is generally associated with increased neuronal vulnerability [34]. However, the basal forebrain cholinergic system is selectively vulnerable in human brain diseases, while the cholinergic cells in the pontine cholinergic system appear to resist neurodegeneration [35,36]. This is emphasized both by in vivo studies demonstrating that basal forebrain cholinergic neurons are more susceptible to toxic agents such as aluminum, nitric oxide, and ethanol as compared to those in striatum and brain stem [37–39]. In cell culture experiments, RN46A neuroblastoma cells from raphe nucleus, when differentiated to cholinergic phenotype appeared to be more susceptible to β-amyloid than those differentiated to serotoninergic phenotype [40]. In this regard, it has to be mentioned that cholinergic cells differ from other neuronal cell types by utilizing acetyl-CoA not only for energy production but also for acetylcholine synthesis. Thus the particular vulnerability and differential susceptibility of cholinergic neurons to various toxic conditions has been suggested to be due to different ratios of their acetyl-CoA energy production and acetylcholine synthesizing capacities. Cholinergic cells appear to have a higher demand for energy production and therefore should respond more sensitive to aging-related energy (glucose) deprivation [41]. Indeed, the comparison of gene expression profiles performed in basal forebrain and pontine cholinergic cells revealed that aging elevates metabolic activity in cholinergic neurons that occurs to a much greater extent in the basal forebrain than in the brain stem (pontine) [42].

2. Cholinergic neurodegeneration and dysfunction in Alzheimer’s disease

Changes in cholinergic system during aging and in AD have been documented by assessing the major functional components of cholinergic cells and signaling, the acetylcholine synthesizing and degrading enzymes, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), respectively, the vesicular acetylcholine transporter (VACHT) to transport acetylcholine into the vesicles, the cholinergic muscarinic (mAChR) and nicotinic acetylcholine receptors (nAChR) for synaptic signaling, as well as the requirement of cholinergic neurons to receive neurotrophic support by NGF mediated through high- (trkA) and low-affinity (p75NTR) receptors for survival.

Evidence of severe deficits of presynaptic cholinergic markers in the cerebral cortex of patients with early-onset of AD was already provided in the late 1970s by a number of studies [43–46]. The correlation of clinical dementia ratings with the reductions in a number of cortical cholinergic markers such as ChAT, mAChR, and nAChR binding as well as levels of acetylcholine [47–49], suggested an association of cholinergic hypofunction with cognitive deficits, which led to the formulation of the cholinergic hypothesis of memory dysfunction in senescence and in AD [50].

While there is no doubt that severe loss of cortical cholinergic innervation exists already in the initial stages of presenile (early
onset) as well as in the advanced stages of late-onset AD, the assumption of cholinergic denervation being an early and initial stage also in mild, late-onset AD has been debated (for reviews, see e.g. [24,51]). Only mild loss of AChE activities has been observed in patients with mild cognitive impairment (MCI, a prodromal stage of AD), and early forms of AD, as revealed by PET studies using ligands that label AChE in vivo [52,53]. Furthermore, in contrast to patients with advanced AD, in autopic brain samples of patients with MCI and early AD no decrease in ChAT activity has been observed in a number of brain regions studied [54,55]. Similarly, the number of ChAT-positive and VACHT-positive cells was unaltered in MCI as compared to non-demented controls [56]. Surprisingly, in hippocampus and frontal cortex of MCI patients, even an increased activity of ChAT has been observed [57,58], indicating that the cognitive deficits observed are seemingly not interrelated with ChAT activity. As a disconnection of glutamatergic entorhinal cortex input to the hippocampus occurs early in the disease [59,60], it has been suggested that the upregulation of hippocampal ChAT in MCI cases may be due to the replacement of denervated glutamatergic synapses by cholinergic input arising from the septum [51]. This is emphasized by animal experimental studies demonstrating reactive sprouting in the hippocampus following perforant path transsections in rats [61]. While the number of ChAT-positive neurons was unchanged, the trkA and p75NTR-containing neurons, which co-localize with ChAT, were significantly reduced in the nucleus basalis of subjects with MCI as compared to those with no cognitive deteriorations. These findings suggest a downregulation of trkA and p75NTR receptors; thus a dysfunction of cholinergic neurons rather than cholinergic cell loss [62]. This is further emphasized by observations that other parameters of cholinergic function such as acetylcholine release, high-affinity choline uptake, expression of mACHRs and nACHRs are also altered in MCI and early AD (see e.g. [63,64]).

Gene expression analysis of single basal forebrain cholinergic neurons revealed that trkA but not p75NTR is reduced in MCI. The NGF precursor, proNGF, has been observed to be increased in the cortex of MCI and AD. As proNGF accumulates in the presence of reduced cortical trkA and sustained levels of p75NTR, a shift in the balance between cell survival and death molecules may occur in early AD. Similarly, BDNF and proBDNF, are reduced in cortex of MCI, further depriving basal forebrain cholinergic cells of trophic support [62]. ProNGF is released from the cerebral cortex in an activity-dependent manner together with enzymes required to generate mature NGF. Thus, the upregulation of proNGF observed in AD may indicate a dysregulation in the maturation of NGF leading to enhanced vulnerability of the cholinergic system in AD [65].

Enhancement of cholinergic neurotransmission by galantamine administration to subjects with MCI has been shown to specifically improve hippocampal function, thus further supporting the suggestion that cholinergic deficits in MCI are functionally relevant [66]. Cholinergic challenges in AD patients and in subjects with MCI differentially affected hippocampal activation as revealed by functional magnetic resonance imaging (fMRI). These data support the suggestion of a key role of the cholinergic system in the functional processes that lead to AD [67]. Malfunction of the cholinergic system may be tackled pharmacologically via AChE inhibitors or mAChR agonists that have been shown to ameliorate the cholinergic deficit at early stages of the disease, and slow-down the progression [68–70].

3. Cholinergic dysfunction in AD: do β-amyloid and tau protein play a particular role?

Degeneration of basal forebrain cholinergic cells has also been observed in a number of other dementing disorders, such as Parkinson’s disease [71–73]. Down-syndrome, progressive supranuclear palsy, Jakob–Creutzfeld disease [74], Korsakoff’s syndrome [75–77], and traumatic brain injury [78,79]. However, in contrast to many other dementing disorders, in AD the cholinergic dysfunctions are accompanied by the occurrence of two major histopathological hallmarks such as β-amyloid plaques deposed extracellularly in cerebral cortical and hippocampal areas, and neurofibrillary tangles that occupy much of the cytoplasm of select cortical pyramidal neurons, provoking the question whether they play a role in inducing or mediating cholinergic dysfunction in AD. It has been hypothesized that β-amyloid peptides induce neurodegenerative changes at cholinergic terminals (see [63,80]). However, it is still a matter of debate whether the loss and degeneration of cholinergic terminals are primary events or secondary to the β-amyloid plaque pathology.

3.1. Evidence from in vivo studies

The most predominant β-amyloid peptides detected in Alzheimer plaques are β-amyloid(1–40) and β-amyloid(1–42). β-Amyloid(1–42) is more fibrillogenic and displays higher neurotoxicity in vivo than β-amyloid(1–40) [81]. The insoluble, high-molecular weight fibrils are major components of the senile plaques in the Alzheimer brain and have been assumed for a long time to be the most toxic forms responsible for cholinergic neurodegeneration. However, recent evidence indicates that soluble oligomers of Aβ (such as low-molecular weight monomers, oligomers and amyloid-derived diffusible ligands (ADDLs), as well as protofibrils represent the main neurotoxic species that lead to early neuronal dysfunction and memory deficits in AD (for reviews, see e.g. [82]). Thus, it has been suggested that β-amyloid oligomers are synaptic-specific ligands, which are able to inhibit hippocampal long-term potentiation in brain slices and rat brains in vivo. In different cell and animal models prefibrillar assemblies of β-amyloid have been shown to induce neurotoxicity, electrophysiological changes and disruption of cognitive function [83], which may explain why early onset of cholinergic dysfunction is in progress before there is considerable plaque formation in AD. Indeed, in Alzheimer brains, it has been observed that the severity of neurodegeneration correlates best with the pool of soluble β-amyloid rather than with the number of insoluble β-amyloid plaques [84]. These data fit well with observations in transgenic mice that express the Swedish double mutation of human APP (Tg2576 mouse) showing a surprisingly minimal correlation among the accumulation of insoluble Aβ peptides, neuronal loss and memory impairments (for references, see also [1,85]). Immunohistochemical and autoradiographic radioligand binding studies in brain sections of developing Tg2576 mice revealed decreases in cholinergic fiber density, in mAChR and nAChR binding levels in the cerebral cortex already before onset of plaque deposition [86–88]. This further provides in vivo evidence of a modulatory role of soluble β-amyloid on cholinergic neurotransmission and may explain the deficits in learning and memory observed in these mice also before significant plaque load occurs [86]. Pharmacological inhibition of muscarinic cholinergic receptors in the cerebral cortex of Tg2576 mice favored the β-secretory path of APP processing, further supporting the role of cholinergic system in APP processing [89].

The synthesis of acetylcholine in cholinergic cells requires the presence of acetyl-CoA which is generated by glucose degradation through glycolysis. To reveal whether β-amyloid plays a role in mediating cholinergic dysfunction by affecting key enzymes of brain glycolysis, expression and activity of the phosphofructokinase (PFK) was studied in the cerebral cortex of aging transgenic Tg2576 mice [90]. In 24-month-old transgenic Tg2576 mouse cortex, but not in 7-, 13-, and 17-month-old mice, the copy number
of PFK-C mRNA, the PFK protein level and PFK enzyme activity was significantly reduced as compared to non-transgenic littermates, while the mRNA level of the other PFK isoforms did not differ between transgenic and non-transgenic tissue samples. In situ hybridization in brain sections from aged Tg2576 mice revealed reduced PFK-C mRNA expression in β-amyloid plaque-associated neurons and upregulation in reactive astrocytes surrounding β-amyloid deposits. The data demonstrate that only long-lasting high β-amyloid burden impairs cerebral cortical glucose metabolism by reducing PFK activity in β-amyloid plaque-associated neurons and concomitant upregulation in reactive, plaque-surrounding astrocytes [90].

Physiological evidence indicates that central cholinergic pathways are also involved in the regulation of cerebral cortical blood flow. The cholinergic axons originating from the basal forebrain project not only to the cortical neuropile but also to arterioles, capillaries and to astrocytes associated with vessels within the cerebral cortex. Heterogeneous mAChRs have been detected in cortical microvessels which are assumed to mediate NO-dependent vessel dilation [91]. An intrinsic microvascular pathology is apparent in the vast majority of AD cases [92,93], thus suggesting a link between β-amyloid production, impairments in cerebrovascular function and basal forebrain cholinergic deficits in AD. A semiquantitative immunohistochemical study in aged Tg2576 mice revealed a β-amyloid-mediated decrease in cholinergic innervation of cortical blood vessels [94], which may contribute to the alterations of the cerebrovascular system observed in transgenic Tg2576 mice [95].

3.2 β-Amyloid affects a specific gene pool in a cholinergic cell line

The cholinergic cell line SN56.B5.G4 created by fusion of N18TG2 neuroblastoma cells with cholinergic neurons derived from septal regions of 21-day-old C57Bl/6 mice has been used as an appropriate model to study the specific susceptibility and vulnerability of cholinergic cells to various neurotoxic signals, and to explore the intracellular metabolic pathways that are mostly affected by pathogenic insults. Thus a number of neurotoxic compounds such as aluminum, nitric oxide, and β-amyloid that were tested for their in vitro cholinotoxicity, demonstrated differential toxicity to cholinergic SN56.B5.G4 cells (for review, see [41]). Single agents with low cytotoxicity could, in combination with other agents, exert harmful effects on neuronal cells by finally inhibiting acetyl-CoA metabolism. The fact that acetyl-CoA is required for both energy generation and acetylcholine synthesis may render cholinergic cells particularly vulnerable and susceptible to particular pathological signals. However, it remains to be elucidated whether changes in acetyl-CoA and energy metabolism are primary events that stimulate further cholinergic cell death or whether these changes represent a final downstream cascade initiated by the action of pathological signals [41].

Using the cholinergic cell line SN56.B5.G4, the effect of different AB(1–42) aggregates on cell viability has been investigated. Exposure of differentiated SN56.B5.G4 cells for 24 h, and 72 h with 50 μM fresh made, oligomeric β-amyloid(1–42) significantly reduced cell survival up to 68% and 55%, respectively, compared to HEPES-treated control cells, while incubation with fibrillar β-amyloid(1–42) preparations did not affect cell viability of SN56.B5.G4 cells, as revealed by MTT reduction assay [96]. Microarray analyses then demonstrated that exposure of SN56.B5.G4 cells by oligomeric β-amyloid(1–42) affected the expression level of a number of genes that were not influenced by non-specific oxidative stress, suggesting that β-amyloid displays a particular toxicity for cholinergic neurons, at least for SN56.B5.G4 cells. Many of the gene products affected by β-amyloid(1–42) exposure were present in the endoplasmatic reticulum (ER), Golgi apparatus and/or otherwise involved in protein modification and degradation (chaperones, ATP6) indicating a possible role for ER-mediated stress in β-amyloid-mediated toxicity. Moreover, a number of genes, which are known to be involved in AD (clusterin, acetylcholine transporter), were found to be affected following β-amyloid(1–42) exposure of SN56.B5.G4 cells [96].

Microarray analyses exhibit a valuable tool for large-scale screening of differential gene expression as a consequence of β-amyloid treatment, but cannot provide information about protein levels, posttranslational modifications of gene products or activity changes, which are important to understand the molecular processes of signal transduction pathways and intracellular interactions between proteins that influence cellular function. Therefore, additionally, proteomic analyses of lysates of SN56.B5.G4 cells exposed to oligomeric β-amyloid(1–42) were performed to identify β-amyloid-mediated changes in protein levels and phosphorylation status, which should provide complementary information to the microarray data obtained recently [96]. The proteomic analyses revealed that the levels of calreticulin, and mitogen-activated protein kinase kinase 6 were upregulated in cholinergic SN56.B5.G4 cells exposed to β-amyloid(1–42), while γ-actin appeared downregulated. β-amyloid(1–42) exposure of cholinergic SN56.B5.G4 cells led to decreased phosphorylation of phosphoproteins, such as the Rho GDI dissociation inhibitor, the ubiquitin carboxyl terminal hydrolase-1, and the tubulin α-chain isotype Ma6, as compared to untreated control lysates (Fig. 1). The proteins identified have also been reported to be affected in brains of AD patients, suggesting a potential role of β-amyloid in influencing the integrity and functioning of the proteome in Alzheimer’s disease [97]. The data further suggest that β-amyloid(1–42) mediates its cytotoxic action by affecting key proteins of a number of physiological processes that also play a role in apoptosis induction in pathological conditions, such as cytoskeletal changes (γ-actin, α-tubulin), protein degradation of misfolded or damaged proteins (calreticulin, UCHL-1), stress-related MAPK signaling (MAPK kinase 6), and GTPase function (Rho GDI).

3.3 β-Amyloid and nAChRs

Alzheimer patients also show a significant reduction in nAChRs which has been documented by Western blotting [98,99], by immunohistochemical analysis [100–102], or by radioligand binding studies [103–110], for a recent review, see [111]. Exposure of PC12 cells to β-amyloid resulted in a significant decrease in nAChRs which led to the suggestion that β-amyloid can damage nAChRs [112,113]. Otherwise, there are reports that β-amyloid may affect nAChRs by direct binding with high-affinity to nAChRs, in particular to the α7 subtype [114–118], while other studies have been unable to confirm these findings [113,119]. β-Amyloid has been observed to act as an agonist of α7 nAChRs [120], mediating the activation of the ERK2 MAP kinase signaling cascade [121,122]. Other groups have reported inhibitory actions of β-amyloid on α7 nAChRs [122–126], which appears to depend on the concentration of β-amyloid; low concentrations can activate, higher concentrations desensitize α7 nAChRs [122]. This compares well with observations in triple transgenic mice overexpressing mutated human APP, presenilin-1 and tau (3xTg-AD), which demonstrate an age-dependent reduction in α7 nAChRs as compared to age-matched non-transgenic mice. The loss of α7 nAChRs is preceded by intracellular β-amyloid accumulation and is restricted to brain regions that develop β-amyloid pathology [117], a finding which mimics the situation detectable in brains of Alzheimer patients. Therefore, parts of the cholinergic deficits produced in Alzheimer’s disease as well as in transgenic APP mice could be attributed to the suppression of cholinergic functions by β-amyloid peptides instead of cell death [127].
Recently, it has been hypothesized that α7 nAChRs may represent the missing link in understanding AD etiopathology [128]. This hypothesis stresses recent experimental findings that nicotinic agonists and β-amyloid may compete for the α7 nAChR binding site, whereas intracellular signaling cascades are activated that control either survival or cell death pathways, respectively (for a comprehensive review, see [111]). Moreover, a recent study supports α7 nAChR as a mediator of β-amyloid-induced pathology in AD by demonstrating that both agonists and antagonists may modulate β-amyloid-induced tau phosphorylation through GSK-3β [129]. Deletion of the α7 nAChR gene in the PDAPP Alzheimer mouse model improved cognitive deficits and synaptic pathology, suggesting that blocking α7 nAChR function could alleviate symptoms of AD and may represent a potential treatment strategy [130].

3.4. β-Amyloid and NGF signaling

In a number of neuroblastoma cell lines including PC12, SK-N-BE, NIH3T3, and SK-N-MC cells, it has been shown that p75NTR increases the susceptibility of these cells to β-amyloid toxicity [131–134], presumably by interacting of p75NTR with β-amyloid [132,134]. These observations have recently been further confirmed by a number of in vivo studies. β-Amyloid injected into the hippocampus of p75NTR−/− knock out mice caused less neuronal death as compared to that observed in wild type (p75NTR+/+) mice [135]. Transgenic Thy1-hAPPLondon/Swe Alzheimer-like mice lacking wild type p75NTR did not display β-amyloid-associated basal forebrain cholinergic neuritic dystrophy and reduced cholinergic cortical fiber density as has been observed in transgenic Thy1-hAPPLondon/Swe mice with intact p75NTR [136]. The data provide evidence that p75NTR may represent a major player in β-amyloid-associated cholinergic deficits, mediated through p75NTR-activated c-Jun N-terminal kinase pathway [137–139]. As the p75NTR is mainly expressed by basal forebrain cholinergic cells, these cells appear to be particularly vulnerable to β-amyloid in AD.

3.5. Tau and cholinergic cells

Neurofibrillary tangles, one of the hallmarks of AD, represent intracellular inclusions formed by aggregates of hyperphosphorylated microtubule-associated tau protein, and they are found in select neuronal populations in AD [140,141]. While in the brains of Alzheimer patients no tau mutations have been described, pathogenic mutations in the tau genes cause frontotemporal dementia [142] suggesting that post-transcriptional alterations in tau gene expression may contribute to the cognitive deficits in AD presumably also by interacting with the cholinergic transmission. Several studies have demonstrated that activation of nAChRs results in a significant increase in tau phosphorylation, whereas nAChR activation may prevent tau phosphorylation [143,144]; for reviews see [111,128]. Nicotine was found to induce tau phosphorylation at those sites that were also hyperphosphorylated in AD, presumably mediated through activation of the α7 subtype of nAChRs [144]. This has been further emphasized by observations in triple transgenic 3xTg-AD mice which develop age-and regional dependent accumulation of both plaques and tangles as well as progressive deficits in cognition [145–147]. Chronic nicotine administration to one-month-old 3xTg-AD mice for five months did not change soluble β-amyloid levels but resulted in a striking increase in phosphorylation and aggregation of tau, which appeared to be mediated by p38-MAP kinase [117].

Cholinergic basal forebrain neurons have been shown to demonstrate tau pathology both in patients with mild cognitive impairment and in Alzheimer patients [58,148–151]. In the adult human brain, six tau isoforms are expressed through alternative splicing of a single tau gene on chromosome 17 [152,153]. Three isoforms contain three tandem microtubule binding repeats, while the other three forms contain four microtubule binding repeats. Cell cycle gene expression profiling revealed a shift in the ratio of three-tandem repeat tau to four-tandem repeat tau in individual human cholinergic basal forebrain neurons within the nucleus basalis and CA1 hippocampal neurons during the progression of AD but not during normal aging [154]. Basal forebrain pretangles and tangles have been observed prior to the pathology in the entorhinal/perirhinal cortex indicating that abnormalities in cortical cholinergic axons and tauopathy within the basal forebrain cholinergic system occur very early in the course of life, and increase in frequency in old age and AD [149,155,156]. In a tau transgenic mouse model, tau pathology has been observed in both hippocampus and basal forebrain, suggesting that tau pathology may participate in cholinergic degeneration [157].
In presenile (early onset), and in the advanced stages of late-onset Alzheimer’s disease (AD), a severe loss of cortical cholinergic innervation has extensively been documented, while in patients with MCI, and early forms of AD, apparently no cholinergic neurodegeneration but a loss of cholinergic function occurs. In particular, imbalances in the expression of NGF, its precursor proNGF, the high and low NGF receptors, trkA and p75NTR, respectively, changes in acetylcholine release, high-affinity choline uptake, altered expression of mAChRs and nAChRs in cholinceptive brain regions have been reported to contribute to the cholinergic dysfunction in MCI and early AD. These observations support the suggestion of a key role of the cholinergic system in the functional processes that lead to AD [67]. In contrast to many other dementing disorders, in AD the cholinergic dysfunctions are accompanied by the formation and deposition of β-amyloid, provoking the question whether β-amyloid may also play a role in inducing or mediating cholinergic dysfunction in AD. Indeed, there is abundant evidence that β-amyloid may trigger cholinergic dysfunction through action on α7 nAChRs by affecting NGF signaling, mediating tau phosphorylation, interacting with acetylcholinesterase, and specifically affecting the proteome in cholinergic neurons (Fig. 2).

![Fig. 2. Changes of cholinergic function with aging that have been assumed to gradually leading to AD. Normal aging is accompanied by a gradual loss of cholinergic function caused by dendritic, synaptic, and axonal degeneration, decrease in trophic support, alterations in gene expression, impairments in intracellular signaling, as well as cytoskeletal transport. In patients with MCI, and early forms of AD, apparently no cholinergic neurodegeneration but a loss of cholinergic function occurs. In particular, imbalances in the expression of NGF, its precursor proNGF, the high and low NGF receptors, trkA and p75NTR, respectively, changes in acetylcholine release, high-affinity choline uptake, altered expression of mAChRs and nAChRs in cholinceptive brain regions have been reported to contribute to the cholinergic dysfunction in MCI and early AD. These observations support the suggestion of a key role of the cholinergic system in the functional processes that lead to AD [67].](image-url)

However, in contrast to many other, dementing disorders, in AD the cholinergic dysfunctions are accompanied by the formation and deposition of β-amyloid, provoking the question whether β-amyloid may also play a role in inducing or mediating cholinergic dysfunction in AD. Indeed, there is abundant evidence that β-amyloid may trigger cholinergic dysfunction through action on α7 nAChRs, by affecting NGF signaling, mediating tau phosphorylation, interacting with acetylcholinesterase, and specifically affecting the proteome in cholinergic neurons.

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