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Review

Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease

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ABSTRACT

Aberrant amyloid-β peptide (Aβ) accumulation along with altered expression and function of nicotinic acetylcholine receptors (nAChRs) stand prominently in the etiology of Alzheimer's disease (AD). Since the discovery that Aβ is bound to α7 nAChRs under many experimental settings, including post-mortem AD brain, much effort has been expended to understand the implications of this interaction in the disease milieu. This research update will review the current literature on the α7 nAChR–Aβ interaction *in vitro* and *in vivo*, the functional consequences of this interaction from sub-cellular to cognitive levels, and discuss the implications these relationships might have for AD therapies.

Contents

1. Introduction

Alzheimer's disease (AD) is marked by selective cholinergic denervation of the cerebral cortex which is most severe in the temporal lobes and the adjacent limbic and paralimbic areas. The hippocampus is a particularly early and vulnerable target of the disease. These neocortical cholinergic pathways are critical for the modulation of attention and memory; as such, the AD cholinergic lesion manifests as episodic memory impairment [1–4]. The clinical observations that cholinomimetics induce symptomatic improvement in AD and the correlation between the magnitude of cholinergic depletion and the severity of dementia provide clinical

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evidence for the relevance of the cholinergic lesion to the clinical features of AD [5–8].

The basal forebrain, including the medial septal nucleus, diagonal band nuclei, and nucleus basalis, is the major source of cholinergic input to the hippocampus and neocortex. The α7 subtype of nicotinic acetylcholine receptors (nAChRs) is particularly enriched in these cholinergic target areas; in fact, initial Aβ deposition in early AD overlap with α7 nAChR expression in the basal forebrain cholinergic system [9,10]. Furthermore, the cholinergic deficit in early AD is due in part to altered expression and function of these receptors [8,11–16]. The α7 nAChRs flux the pluripotent second messenger Ca^{2+} and have been shown to modulate neuron excitability, neurotransmitter release, the induction of LTP, learning, and memory [17–21]. Likewise, in patients with mild to moderate AD, activation of this receptor improves attention, learning, and memory performance [22–26]. Therefore α7 nAChRs are highly implicated in the etiology of early AD.

In the decade-plus since the discovery of a high-affinity interaction between Aβ peptides and α7 nAChRs, several investigative teams have aggressively pursued the biological relevance of this interaction. At this time, these efforts support a model in which the α7 nAChR–Aβ interaction performs a physiologic role since Aβ peptides are continuously produced under normal conditions as well as contributes to the etiology of AD as Aβ peptide concentration and aggregation proceed pathologically [9,10,27–30]. This research update will discuss the current literature on the α7 nAChR–Aβ interaction *in vitro* and *in vivo*, the functional consequences of this interaction from subcellular to cognitive levels, and discuss the implications these relationships have for AD therapies.

2. Aβ peptides are conformationally dynamic

In vivo generated Aβ fragments can be of different lengths and can take many forms, all of which may behave differently in biological systems. Aβ in a monomeric form is relatively unstructured *in vitro*. Oligomerization (dimers, trimers, tetramers, hexamers, dodecamers, etc.) can make the fragment more rigid while retaining its aqueous solubility. Further aggregation of Aβ can create an insoluble fibril structure, which is a key component of the amyloid plaques found in individuals with AD. While it is widely agreed that purely monomeric and fibrillar assemblies of Aβ peptide are unlikely to be the disease-relevant stoichiometries, which oligomeric aggregate species is responsible for the synaptic dysfunction and ultimate neurodegeneration in AD remains debated.

Several lines of evidence indicate that oligomeric assemblies of Aβ possess unique functional properties including the ability to modulate synaptic transmission and influence learning and memory in an α7 nAChR-dependent manner. For example, purified oligomers (dimers, trimers, as well as a 56 kDa dodecamer aggregate) of *in vitro* and *in vivo* produced Aβ can disrupt synaptic plasticity and cognitive function when administered at high (nanomolar) concentration and α7 nAChR activation can overcome LTP impairments suggesting that α7 nAChRs are an important target of oligomeric Aβ [31–35]. However, recent work has implicated very low (picomolar) concentrations of monomer, trimer, tetramer, and hexamer $Aβ_{1–42}$ as playing a role in modulating hippocampal synaptic plasticity and enhancing cognitive function in mice via an α7 nAChR-dependent mechanism [36,37]. How can one observe such conflicting effects of α7 nAChR–Aβ interaction? Given that Aβ peptide structure and aggregation properties are dynamic and depend on concentration, pH, salinity, chelation status, and temperature, it is not surprising that very different results are obtained with Aβ solutions prepared in methodologically distinct ways.

The work reviewed here exclusively used soluble Aβ peptides that likely represent a mixture of monomeric and oligomeric assemblies. However the precise structure and aggregation state of the peptide solution in these studies is largely unknown, further confounding the interpretation of results. Therefore, it will continue to be important to not only specify the concentration of soluble Aβ used, but also to specify the structural nature of the preparation. This is currently becoming much more of a trend and several recent publications have attempted to structurally define the peptide solution [36,38–40]. These efforts greatly facilitate our interpretation of investigations into the complex nature of Aβ peptide interaction with α7 nAChRs. It is hoped that future work will correlate the effects of the Aβ–nAChR interaction with specific peptide structures; as has been formally requested in a recent editorial [141].

3. α7 nAChRs and Aβ interact with high-affinity *in vitro* and *in vivo*

An α7 nAChR–Aβ interaction was first described over a decade ago; since then many studies have reported seemingly incongruent consequences of this interaction emphasizing a complex biology that underlies this interaction. Initial work published by Wang et al. [27,28] demonstrated that α7 nAChRs and Aβ are co-localized in AD cortical regions including the hippocampus; these proteins are found not only in the membrane fraction but also in amyloid plaque deposits. They also demonstrated that the receptor–peptide complex could be co-immunoprecipitated and detected with immunoblot; this was shown for both control and AD brain samples. Because this association resists detergent treatment, it prompted the investigators to postulate that α7 nAChRs and Aβ may associate with rather high-affinity, possibly for extended periods of time [27,41]. As discussed in the following sections, this hypothesis has yet to be refuted and more recent findings indicate that Aβ-mediated inactivation of α7 nAChRs may be one of the detrimental aspects of this protein interaction in AD [9,10,42].

3.1. Nature of Aβ binding to α7 nAChRs

The exact nature of the Aβ interaction with α7 nAChRs is not well understood. Computer simulated docking studies have been performed by Espinoza-Fonseca [43] utilizing the homology model of the human α7 nAChR derived from the X-ray structure of the acetylcholine-binding protein (AChBP) and the lowest energy NMR structure of the human $Aβ_{1–42}$ peptide as well as four fragments (amino acids 1–11, 10–20, 12–28, and 22–35) [43,44]. These analyses were achieved using a modified version of ESCHER software that analyses the complementarity of the target and probe proteins in 360° using their solvent accessible surfaces. Their results indicated that the full length peptide and peptide fragments bind parallel to the receptor within the binding interface between two subunits. Based on the series of docking studies with the full length Aβ peptide and the four fragments in complex with the α7 nAChR, Espinoza-Fonseca concluded that the interaction domains common amongst all five receptor: peptide complexes involved residues V12-K28 of $Aβ_{1–42}$ and the agonist binding loop C of one subunit of the receptor and a loop delineated by amino acids 62–74 and loop G of the adjacent subunit of the receptor. Thus, it appears energetically favorable for monomeric $Aβ_{1–42}$ to bind components of the agonist binding site.

To further support the strength of these studies, the fragments used in the Espanoza-Fonseca studies correspond to the fragments identified by Wang et al. [45] that were most effective in competition binding studies against cells expressing α7 nAChRs. The $Aβ_{1–42}$ peptide fragments defined as interaction points with the ligand binding domain of the α7 nAChR are common between

both $A\beta_{1-40}$ and $A\beta_{1-42}$ suggesting that the two additional hydrophobic amino acids at the C-terminus of $A\beta_{1-42}$ alter the conformation of the central hydrophilic portion of the peptide to increase binding affinity [28].

Initial competition binding studies on membrane preparations from brain regions and cell lines expressing the α7 nAChR indicated that Aβ association occurred with an affinity in the low picomolar range while similar experiments for α4β2 nAChRs indicated an affinity 100–5000 times lower [27,28]. The apparent affinity for the α7 nAChR–$A\beta_{1-42}$ interaction and the fact that *soluble* $A\beta_{1-42}$ in healthy brain and CSF has been estimated at picomolar values indicates that these two proteins could associate under normal physiologic conditions leading to receptor activation [46–48]; recent behavioral and synaptic plasticity studies provide evidence that this is indeed the case, at least in hippocampus [36,37]. As AD progresses Aβ levels exponentially increase in AD-affected brain regions, achieving nanomolar levels and, as discussed in later sections, likely lead to α7 nAChR inactivation [46–48].

Curve fit analysis from binding studies on α7-expressing cells, suggested that there were two saturable Aβ binding sites [28]. While extensive binding studies with Aβ peptides and α7-expressing cells have not since been published, several functional studies utilizing a variety of preparations have pharmacologically blocked Aβ-mediated effects via α7 nAChR-selective antagonists such as α-bungarotoxin (BTX) and methyllycaconitine (MLA) [31,36,39,49–54]. These publications utilized $A\beta_{1-42}$ preparations that were either roughly defined as 'oligomeric' or were more precisely defined as being comprised of a mixture of at least two of the following: monomer, dimer, trimer, and hexamer. These data suggest that Aβ preparations that result in α7 nAChR activation utilize a binding site that is comprised, at least in part, by components of the Cys-loop inter-subunit agonist binding site.

It should be noted that a number of those that have observed Aβ-mediated functional antagonism of α7 nAChRs have reported that this is noncompetitive in nature since α7 nAChR-selective antagonists such as BTX or MLA were unable to block, or agonist was able to only partially overcome Aβ inhibitory effects [55–58]. These noncompetitive binding results infer that the Aβ preparations utilized (uncharacterized in these studies) gained access to a binding site distinct from the Cys-loop inter-subunit acetylcholine ligand binding pocket.

A recently described intra-subunit allosteric binding pocket located within the transmembrane domain of the α7 nAChR provides a potential structure-function mechanism to explain noncompetitive Aβ antagonism [59–61]. In support of this model, recent work on heterologously expressed α4β2 and α2β2 nAChRs showed that the positive allosteric modulator desformylflustrabromine relieves the noncompetitive $A\beta_{1-42}$ blockade [62]. It will be an important (and challenging) effort to delineate the structural and conformational parameters that yield competitive and noncompetitive Aβ–α7 nAChR interaction.

4. The nAChR–Aβ interaction leads to receptor activation and receptor inhibition

The evidence for an inhibitory versus a stimulatory role for Aβ on α7 nAChRs is fairly equally divided in the literature (Table 1). Superficially, these reports appear contradictory; however potential underlying issues regarding the origin of the receptor populations and specifics of the biological preparations as well as detection methods differ amongst laboratories will be discussed. Differing effects of Aβ on α7 nAChRs indicate that the details regarding cell type, sub-cellular location, subunit stoichiometry, accessory protein population, lipid composition, and post-translational modifications of the receptor may significantly influence receptor properties; as has been shown many times previously for this receptor class [63–67]. In these studies, an equally important, but often overlooked variable is the Aβ peptide itself. As discussed above, early investigations did not characterize the conformation and aggregation state of the Aβ peptide stock solutions utilized. However this is changing; some studies discussed below included this information and will be discussed in terms of the results obtained.

4.1. α7 nAChR antagonism

The first indication that $A\beta_{1-42}$ and nAChRs *functionally* interact demonstrated that $A\beta_{1-42}$ inhibited nAChR currents recorded from GABAergic interneurons in acutely prepared rat hippocampal slices [68]. Caged-carbachol-induced currents measured with whole-cell recordings were maximally inhibited (39%) with a dose of 500 nM peptide; doses as low as 100 nM were effective. These effects were rapidly reversible under whole-cell recording conditions. One of the Aβ-sensitive channels they characterized using patch clamp recordings was sensitive to the α7-selective antagonist MLA. Thus, rat hippocampal interneurons possess α7 nAChRs that are reversibly antagonized by $A\beta_{1-42}$ via a mechanism that decreases the probability of opening.

Subsequent work from this same group studied $A\beta_{1-42}$ effects on nAChRs expressed in *Xenopus* oocytes [57]. Co-application of a maximal dose of carbachol and 1 μM $A\beta_{1-42}$ to oocytes expressing rat α7 nAChRs resulted in no effect on α7 nAChRs currents in contrast with their previous findings in hippocampal interneurons; further suggesting that the *in situ* environment, such as the cell system utilized for expression, can significantly alter receptor properties [69,70].

Table 1
Abeta effects via alpha7 nAChRs.

nAChR subtype	Experimental preparation	Type of interaction	Possible mechanism/ downstream consequence	Reference
Human α7	Human brain: control and AD	Co-localization and Co-IP	High-affinity interaction	[32]
Human α7	Cell culture	High-affinity binding	Competitive binding (BTX, Aβ)	[33]
Human α7	*Xenopus* oocytes	Functional antagonism (reversible)	Noncompetitive (ACh)	[63]
Human α7	*Xenopus* oocytes	Functional antagonism (reversible)	Noncompetitive (ACh)	[60]
Human α7	SY5Y neuroblastoma cells	Receptor activation	ERK MAPK activation	[44]
Rat α7, non-α7	Acute hippocampal slice, GABAergic interneurons	Functional antagonism (reversible)	Decreased open p_o	[72]
Rat α7	Cultured hippocampal neurons	Functional antagonism (reversible)	Noncompetitive (ACh)	[75]
Rat α7	*Xenopus* oocytes	Receptor activation	Ca^{2+}-influx	[55,59]
Rat α7	*Xenopus* oocytes	No effect	N/A	[62]
Rat, mouse α7	Isolated presynaptic terminals	Receptor activation	Increased presynaptic $[Ca^{2+}]$	[57,58]
Mouse α7	Cultured hippocampal and cortical neurons	Receptor activation	Akt phosphorylation	[81]
Rat, mouse α7β2	Acute basal forebrain slice, *Xenopua* oocytes	Receptor antagonism	Reversible	[75]
α7 knockout mice	Isolated presynaptic terminals	Absence of receptor activation	Loss of increased presynaptic $[Ca^{2+}]$	[77]

α7 nAChR antagonism was also observed in rat hippocampal neuronal cultures, it was demonstrated that the response of both somato-dendritic and presynaptic α7 nAChRs was rapidly and almost completely blocked by exposure to 100 nM $A\beta_{1-42}$ and they reported an IC_{50} value of 7.5 nM [56]. Full recovery occurred within 5 min of washout. This functional antagonism appeared noncompetitive from [^{125}I]-BTX binding assays. Additional experiments demonstrated that Aβ blockade was voltage-independent, did not result from open channel block, and likely resulted from interaction with the N-terminal extracellular domain of the receptor. Furthermore, it was determined that intracellular Ca^{2+} and G-protein activity was not necessary for inhibition of α7 nAChR function by $A\beta_{1-42}$, suggesting that prior receptor activation did not mediate antagonism.

Pym et al. [58] expressed human α7 nAChRs in *Xenopus* oocytes and found these receptors to be antagonized by $A\beta_{1-42}$ (and $A\beta_{1-40}$). Maximum acetylcholine currents were inhibited approximately 50% in the presence of 10 nM of either peptide. Given that binding studies indicated that $A\beta_{1-42}$ exhibits higher affinity than $A\beta_{1-40}$ suggests that, at this concentration, the dose–response curves over lap. Concentrations in the range of 1 pM–100 nM were tested and failed to activate the receptor. Similar to the above described studies, Pym et al. [58] found that Aβ effects were reversible. Grassi et al. [55] also reported that human α7 nAChRs expressed in *Xenopus laevis* oocytes were antagonized by 100 nM $A\beta_{1-42}$. Antagonism was noncompetitive and an analysis of a dose–response study for inhibition of currents elicited by 100 μM ACh yielded an IC_{50} value of 90 nM. Attempts to activate these receptors with 10 nM $A\beta_{1-42}$ were unsuccessful. Taken together, this set of studies suggests that soluble (human) Aβ does not activate human α7 nAChRs and are noncompetitively antagonized by Aβ peptides. Again it must be emphasized that the conformation and aggregation state of the Aβ preparations used in the studies above is unknown.

A contrasting result was recently obtained when human neuroblastoma cells that express α7 nAChRs were exposed to human oligomeric $A\beta_{1-42}$ [39]. The results from Young et al. [39] were very similar to a series of studies from Dineley's group utilizing rat hippocampal slices and both human and rat oligomeric $A\beta_{1-42}$ [38,50]. Concentrations in the picomolar to nanomolar range of oligomeric $A\beta_{1-42}$ applied to SY5Y neuroblastoma cells resulted in ERK MAPK activation and this was blocked by the competitive antagonists MLA and BTX [39]. These findings again emphasize that the conformation and aggregation state of the Aβ peptide preparation is a critical factor in the study design.

The results discussed above were obtained from homomeric α7 nAChRs based upon receptor kinetics and pharmacological properties (*in situ* recordings) or through heterologous expression in *Xenopus* oocytes, for example. An α7-containing heteromeric nAChR was described a short time ago by Drs. Wu and Lukas in basal forebrain cholinergic neurons [71]. In this study, it was demonstrated that nAChRs expressed on freshly dissociated cholinergic medial septum/diagonal band (MS/DB) neurons exhibit mixed kinetic and pharmacological properties of α7- and β2-containing nAChRs. These 'mixed' properties were absent in MS/DB neurons prepared from β2 nAChR knockout mice. Functional antagonism of α7β2 nAChRs expressed on cholinergic MS/DB neurons was demonstrated at $A\beta_{1-42}$ concentrations as low as 1 nM; 100 pM was ineffective. Additionally, it was demonstrated that oligomeric $A\beta_{1-42}$ more effectively antagonized these receptors than fibrillar $A\beta_{1-42}$; monomeric $A\beta_{1-42}$ had no effect. Such selective sensitivity to relatively low concentrations of oligomeric $A\beta_{1-42}$ and the necessity of nAChR function in maintenance of the cholinergic phenotype suggests that the selective vulnerability of the basal forebrain cholinergic system during early AD may in part be due to blockade of this heteromeric α7β2 nAChR by oligomeric Aβ assemblies occurring due to AD progression.

In all cases discussed above in which functional antagonism was reported, inhibition required at least 1–10 nM Aβ and block was typically incomplete, but at least 30%. These studies are best summarized as rat and human α7 nAChR inhibition by $A\beta_{1-42}$ peptide required pre-application of the peptide; receptor inhibition was reversible and exhibited noncompetitive binding properties [55–58,68,72].

4.2. α7 nAChR activation by oligomeric assemblies of $A\beta_{1-42}$

While an interaction between α7 nAChRs and Aβ peptide is well-established, in the presence of Aβ, α7 nAChRs rapidly desensitize making direct electrophysiological recordings a challenge. Rat α7 nAChRs expressed in *Xenopus* oocytes were activated following application of femtomolar to nanomolar concentrations of $A\beta_{1-42}$ [54]. Analysis of the $A\beta_{1-42}$ preparation by non-denaturing Tris-Tricine gel electrophoresis indicates that the Aβ used in these experiments was primarily hexameric oligomers with additional components of trimers and monomers [38]. Receptor activation led to Ca^{2+} influx as evidenced by a reduction in current amplitude when Ca^{2+} in the recording solution was replaced by Ba^{2+}, thus preventing the activation of the endogenous Ca^{2+}-activated chloride current that enhances membrane depolarization and current amplitude. $A\beta_{1-42}$ activation of α7 nAChRs was blocked by the α7-selective antagonist MLA and cross-desensitized by the α7-selective agonist DMXB [3-(2,4-dimethyoxybenzylidene)-anabaseine], suggesting that the α7 nAChR ligand binding domain and the $A\beta_{1-42}$ binding site at least partially overlap. The lowest doses that were effective in this study (100 fM–10 pM) and the observation that $A\beta_{1-42}$ was more potent in activating α7 nAChRs than $A\beta_{1-40}$, are consistent with the binding studies performed by Wang et al. [28] in which it was observed that α7 nAChRs exhibit higher affinity for $A\beta_{1-42}$ versus $A\beta_{1-40}$. While it was demonstrated that Aβ peptides could directly activate α7 nAChRs; these currents were small (~200 nA). Furthermore, high doses or prolonged exposure to $A\beta_{1-42}$ led to receptor inhibition, possibly through a desensitization mechanism [54]. This was suggested by the inverted U shape of the dose–response curve and the observation that more than one exposure or pre-exposure to $A\beta_{1-42}$ led to α7 nAChR inactivation. In summary, rat α7 nAChRs expressed by *Xenopus* oocytes are responsive to physiologically relevant doses of oligomeric $A\beta_{1-42}$; however, these currents are relatively small indicating that oligomeric $A\beta_{1-42}$ appears to be highly desensitizing leading to receptor inactivation.

One strategy to overcome the desensitizing nature of $A\beta_{1-42}$ for α7 nAChRs and gain signal-to-noise in measurements of receptor activation is to exploit the high Ca^{2+} permeability of these receptors. As such, α7 nAChR activation commonly leads to Ca^{2+}-induced Ca^{2+} release (CICR); voltage-gated Ca^{2+} channels are another component of the overall intracellular Ca^{2+} signal in cases where nAChRs evoke significant changes in membrane potential. The Nichols' laboratory took advantage of this circumstance by utilizing confocal imaging in combination with fluorescent Ca^{2+}-indicator dyes to record increases in intracellular Ca^{2+} in isolated presynaptic nerve endings purified from rat hippocampus and neocortex [52]. These efforts determined that picomolar $A\beta_{1-42}$ directly led to sustained increases in presynaptic Ca^{2+} via nAChRs. The effect of $A\beta_{1-42}$ was sensitive to BTX, mecamylamine (MEC), and dihydro-β-erythroidine (DHβE), indicating the involvement of both α7-containing and non-α7-containing nAChRs. Interestingly, it was discovered that α7-containing nAChRs are largely involved in the presynaptic actions of Aβ at picomolar concentrations whereas higher nanomolar concentration of Aβ involves mainly

non-α7-containing nAChRs. Prior exposure of these preparations to Aβ occluded subsequent nicotine-evoked increases in presynaptic Ca^{2+}. This and the fact that nicotine, albeit at relatively high concentration, could overcome the occlusion effect of $A\beta_{1-42}$ suggested that the Aβ and the α7 nAChR ligand binding site significantly overlap. Subsequent studies utilizing presynaptic terminals isolated from the hippocampus and cortex of nicotinic receptor knockout mice for either the α7 or β2 nAChR subunit, determined that Aβ-mediated increases in intracellular Ca^{2+} were mainly mediated by β2-containing nAChRs in the hippocampus and α7 nAChRs in the cortex [53,73]. Perhaps the species difference between the two studies underlies the failure to detect α7 nAChR presynaptic involvement in the hippocampus of mice. However, yet another study from this group utilized a neuroblastoma cell line (NG 108–15) transfected with mouse α7 nAChR cDNA found that picomolar–nanomolar soluble $A\beta_{1-42}$ induced increased intracellular Ca^{2+} within axonal varicosities that was blocked by the α7 nAChR antagonist, BTX [73]. Interestingly, cholesterol depletion with methyl-β-cyclodextrin significantly attenuated these responses, suggesting that Aβ-sensitive α7 nAChRs reside within lipid rafts at presynaptic sites. These findings further reinforce that the lipid composition surrounding nAChR receptor transmembrane domains is an important variable contributing to nAChR functional profiles [74,75].

All in all, these studies demonstrate that low concentrations (femtomolar-picomolar) of oligomeric (e.g., trimer, hexamer) $A\beta_{1-42}$ can activate α7 nAChRs *in situ* and heterologously expressed in *Xenopus* oocytes. Receptor activation increases intracellular Ca^{2+} and can potentiate neurotransmitter release. However, higher $A\beta_{1-42}$ concentrations lead to receptor inactivation, likely through a desensitization mechanism. These results put forth the possibility that, under normal physiologic conditions, Aβ and α7 nAChR interaction could lead to receptor activation *in vivo*, as has been recently demonstrated by Puzzo et al. to be discussed in the next section [36,37].

5. Functional consequences of the α7 nAChR–Aβ interaction

Activation of nAChRs causes membrane depolarization and, directly or indirectly, increases the intracellular Ca^{2+} concentration. Thus, when nAChRs are expressed on presynaptic membranes their activation generally increases the probability of neurotransmitter release. When expressed on postsynaptic membranes, nAChR-initiated increases in intracellular Ca^{2+} and depolarization activate intracellular signaling mechanisms that contribute to neuron homeostasis, synaptic plasticity, learning and memory (for review, see [76]). As is the case for receptor activation by ACh or nicotine, Aβ activation of α7 nAChRs runs the gamut of these responses.

5.1. Signal transduction consequences of the α7 nAChR–Aβ interaction

Since the discovery of an α7 nAChR–Aβ interaction several groups have mapped out some of the downstream consequences of this association: Ca^{2+} influx, ERK MAPK activation via the PI3K pathway that results in CREB phosphorylation in both a PKA- and Rsk2-dependent manner [38,39,50,52,54,77]. The studies by Dineley et al. [50] and Bell et al. [38] were performed on organotypic hippocampal slice cultures; specificity of the effects occurring via α7 nAChRs was demonstrated with the α7-selective antagonists MLA and BTX. ERK activation occurred rapidly and at concentrations as low as 10 pM.

Extended exposure to high (nanomolar) concentration of $A\beta_{1-42}$ led to down-regulation of ERK MAPK activity; this is also observed in hippocampal samples from aged Tg2576 in which Aβ is produced in excess from young adulthood onward [38,50]. Interestingly, extended exposure to nanomolar $A\beta_{1-42}$ up-regulates α7 nAChRs in hippocampal cultures, comparable to the effects of chronic exposure to nicotine [50]. Likewise, in the Tg2576 hippocampus α7 nAChRs continue to up-regulate with age as Aβ accumulates, providing further evidence that Aβ and α7 nAChRs interact *in vitro* and *in vivo* [50,78]. Dysregulation of α7 nAChRs, ERK MAPK, and the downstream transcription factor CREB in the hippocampus of Tg2576 mice occurs concomitant with the onset of hippocampus-dependent learning and memory impairments [50]. These combined *in vitro* findings and *in vivo* observations suggest that, in hippocampus, physiological concentrations of $A\beta_{1-42}$ impinge upon signal transduction cascades important for cell homeostasis, synaptic plasticity, learning and memory. Short exposure times (minutes) and moderate concentrations (picomolar–low nanomolar) do not lead to permanent changes in α7 or the ERK MAPK cascade; higher doses and extended exposure time lead to dysregulation of α7, ERK MAPK, and CREB accompanied by learning and memory impairments.

Young et al. obtained very similar results to those from Dineley's group utilizing human SY5Y neuroblastoma cells exposed to human oligomeric $A\beta_{1-42}$. Oligomeric Aβ application resulted in ERK MAPK activation and this was blocked by the α7 nAChR competitive antagonist, MLA, and U0126 compound that inhibits the ERK MAPK upstream kinase, MEK [39].

Utilizing primary neuronal cultures prepared from mouse cortex and hippocampus Abbot et al. demonstrated that acute exposure to nanomolar (4 0 0) $A\beta_{1-42}$ leads to Akt phosphorylation via α7 nAChRs [77]. Akt is closely associated with PI3K activation which itself is involved in signal transduction pathways necessary for neuroprotection as well as synaptic plasticity, learning and memory [79]. nAChRs have long been implicated as playing a role in each of these processes; in neuron models for α7 nAChR-mediated neuroprotection, activation of the PI3K–Akt pathway is a crucial downstream effector of nicotine-induce anti-apoptotic signaling [80–82]. We previously discussed the work of Dineley et al. and Bell et al., in which acute exposure of organotypic hippocampal slice cultures to picomolar–nanomolar $A\beta_{1-42}$ (and

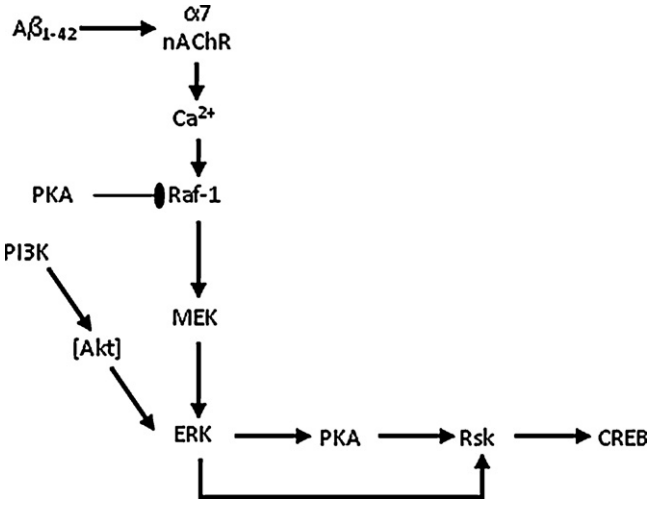

Fig. 1. Summary of the signal transduction consequences of Aβ activation of α7 nAChRs. $A\beta_{1-42}$ acting through α7 nAChRs activates PKA downstream of ERK MAPK PI3K (and Akt) is an intermediary between α7 nAChR activation and ERK MAPK phosphorylation since LY294002 blocked ERK MAPK activation following $A\beta_{1-42}$. ERK MAPK activation leads to p90 Rsk and CREB phosphorylation; $A\beta_{1-42}$-induced p90 Rsk phosphorylation is carried out by both ERK and PKA since U0126 completely obliterated p90 Rsk phosphorylation and H-89 partially reduced it. Adapted from [43,55,64,81].

nicotine) led to ERK MAPK activation via α7 nAChRs [50]. Bell et al. [38] further showed that $A\beta_{1-42}$ (and nicotine) couples to ERK via PI3K in an α7 nAChR-dependent manner. Abbott et al., however, did not test whether PI3K activity was necessary for the Akt phosphorylation observed following acute $A\beta_{1-42}$ exposure; thus there may exist subtle molecular differences between the two systems [77].

To summarize, α7 nAChRs acutely exposed to $A\beta_{1-42}$ leads to activation of signal transduction cascades associated with neuroprotection, synaptic plasticity, learning and memory in an α7 nAChR-dependent manner (Fig. 1). The concentration range that was effective (picomolar–nanomolar) suggests that endogenous Aβ may serve a modulatory role in synaptic transmission, plasticity, and even neuroprotection [36,46,47,83–85]. Further discussion on this topic will be covered in the next section.

The fact that α7 nAChRs also reside on glial cells, notably astrocytes, immediately implies a major physiological role in astrocytic function, since α7 nAChRs flux Ca^{2+}, and changes in intracellular Ca^{2+} are the basis of astrocytic "excitability" [86–88]. These observations clearly imply that the relationship between nAChRs and Aβ is a dynamic one and relies on several factors such as the *in situ* environment in which the nAChR is expressed (somatic, dendritic, presynaptic; neuronal, astrocytic, microglial) as well as the *in situ* status of Aβ (concentration, aggregation state, regional distribution).

5.2. Neurotransmission, synaptic plasticity, learning and memory

The Nichols' laboratory has performed several studies investigating the ability of soluble $A\beta_{1-42}$ to influence neurotransmitter release through activation of presynaptic nAChRs [51,52,73]. Initial studies demonstrated that soluble $A\beta_{1-42}$ activates nAChRs on presynaptic terminals isolated from rat neocortex and hippocampus [52]. The observations that Aβ-induced nAChR activation led to elevation of presynaptic Ca^{2+} and that prior activation of presynaptic nAChRs attenuated subsequent responses to $A\beta_{1-42}$ suggested that this interaction could lead to altered synaptic transmission; however this study by Dougherty et al. did not directly test that outcome.

To address whether presynaptic α7 nAChR–Aβ interaction impinges upon neurotransmission, the same group then utilized the well-established paradigm for nAChR-mediated dopamine (DA) release from prefrontal cortex to test the hypothesis that Aβ-activation of presynaptic nAChRs could lead to neurotransmitter release *in vivo* [51,89]. It has been shown previously that both α7-containing and α4β2 nAChR subtypes are involved in prefrontal cortex DA release; in this study, soluble Aβ was perfused into mouse prefrontal cortex and the effect on the release of DA outflow via microdialysis was assessed [90,91]. In the presence of tetrodotoxin, $A\beta_{1-42}$ at 100 nM evoked the release of DA to ~170% of baseline. $A\beta_{1-42}$-evoked DA release was sensitive to antagonists of α7 nAChRs and was absent in mice in which the α7 nAChR subunit had been genetically deleted, but was intact in mice harboring a null mutation for the β2 nAChR subunit [92,93]. Very low relative concentrations (picomolar) of $A\beta_{1-42}$ caused a slowly developing and long-lasting depression of DA outflow in the prefrontal cortex. Given that the $A\beta_{1-42}$ in these studies was delivered through reverse dialysis, the time to achieve maximal dose is unknown, but likely on the minutes' time scale; therefore, picomolar $A\beta_{1-42}$ may evoke a low sustained level of presynaptic Ca^{2+} rise, leading to synaptic depression. It will be of interest if fast delivery of such a concentration of $A\beta_{1-42}$ has the same effect. Nonetheless, the cumulative work of Dougherty et al. [52] and Wu et al. [51] provide compelling evidence that $A\beta_{1-42}$, most likely acting through α7 nAChRs, alters neurotransmitter release and transmission at certain cortical synapses.

In addition to directly modulating neurotransmitter release, $A\beta_{1-42}$ has additional synaptic effects by modulating NMDA receptor function through a trafficking mechanism. In this study, cortical neuron cultures were used as a cellular model to study glutamatergic synapses and it was found that exposure to high (μM) concentrations of $A\beta_{1-42}$ for prolonged periods of time (≥30 min), led to NMDA receptor endocytosis in an α7 nAChR- and Ca^{2+}-dependent manner [31]. The mechanism involved Ca^{2+}-dependent activation of protein phosphatase 2B that led to striatal-enriched phosphatase (STEP) dephosphorylation and activation, which in turn resulted in dephosphorylation of the NR2B NMDA receptor subunit on a tyrosine residue (Tyr1472, a STEP target). NR2B Tyr1472 dephosphorylation correlated with receptor endocytosis and depression of NMDA-evoked currents in cortical neuron cultures.

Modeling synaptic deficits of early AD by infusing 300 pmoles of $A\beta_{1-40}$ per day for 11–14 days into rat hippocampus followed by *in vivo* HFS-induced LTP at Schaffer collateral–CA1 synapses Chen et al. found that α7 nAChRs were involved in $A\beta_{1-40}$-induced depression of synaptic transmission and deficits in LTP [35,94]. Utilizing the α7 nAChR partial agonist, DMXB, it was demonstrated that DMXB induced EPSPs were impaired in $A\beta_{1-40}$ infused rat hippocampus [95]. In addition, $A\beta_{1-40}$ infused rats also demonstrated impaired LTP that was rescued with DMXB. Control experiments demonstrated that: (1) DMXB enhanced *in vivo* recorded EPSPs in untreated rats; (2) DMXB enhanced EPSPs were blocked by BTX but not DHβE, an α4β2 nAChR antagonist; (3) blocking α7 nAChRs with BTX and MLA (but not α4β2 nAChRs with DHβE) blocked *in vivo* LTP; (4) BTX blocked DMXB enhancement of LTP. Finally, evaluation of input/output curves as well as post-tetanic potentiation and paired-pulse facilitation suggested that $A\beta_{1-40}$ infusion leads to diminished presynaptic Ca^{2+} influx that led the authors to propose a model in which reduced EPSP in $A\beta_{1-40}$ infused rats arises from a decline in presynaptic glutamate release due to α7 nAChR dysfunction. Collectively, the studies by Chen et al. suggest that Aβ-induced blockade of α7 nAChRs can negatively affect synaptic plasticity and, by extrapolation, possibly learning and memory processes [35,94].

Gu and Yakel published additional exciting *in vivo* evidence that Aβ at high (nanomolar) concentration interacts with presynaptic septal cholinergic α7 nAChRs to affect Schaeffer collateral (SC) to CA1 plasticity [40]. Septal cholinergic stimulation was achieved either by electrical stimulation or via an optogenetic approach. The type of plasticity depended upon the timing of septal cholinergic stimulation relative to the SC input; cholinergic input activated 100 ms or 10 ms prior to SC stimulation resulted in α7 nAChR-dependent long-term potentiation (LTP) or short-term depression, respectively. Plasticity was blocked by the α7 nAChR antagonist MLA and absent in α7 nAChR knockout mice; the α4 nAChR-selective antagonist DHβE had no effect. Moreover, these two forms of α7 nAChR-dependent plasticity were disrupted by either 10 nM or 100 nM (but not 1 nM) Aβ exposure suggesting again that inactivation of α7 nAChRs has negative effects on synaptic plasticity.

In a series of studies utilizing low (picomolar) concentration of oligomeric Aβ, Puzzo et al. have developed the idea that endogenous Aβ serves as a positive modulator of hippocampal synaptic transmission via interaction with (presumably) presynaptic α7 nAChRs [36,37]. At the outset, they demonstrated that 200 pM $A\beta_{1-42}$ enhanced Schaffer collateral–CA1 LTP using theta-burst stimulation; enhanced LTP was not achieved if tetanus was not applied nor when a scrambled peptide was perfused with tetanus, and was absent in hippocampal slices prepared from α7 nAChR null-mutant mice. Investigations into the mechanism of the Aβ-induced enhancement of LTP ruled out that Aβ does not affect spontaneous neurotransmitter release; nor did it affect NMDA or

AMPA receptor currents. The authors concluded that 200 pM $A\beta_{1-42}$ increases neurotransmitter release in an α7 nAChR-dependent manner during the tetanus. These results are consistent with a presynaptic effect of Aβ on α7nAChRs. However, since astrocytic Ca^{2+} elevations lead to gliotransmitter release, which can then contribute to synaptic strengthening in the dentate gyrus [96], it is possible glial α7 nAChRs contribute to synaptic modulation and plasticity. This potential scenario will be discussed in the next section.

In the same set of studies, Puzzo et al. [36] report that 200 pM $A\beta_{1-42}$ delivered through bilateral cannulae to the dorsal hippocampus also enhances baseline learning and memory in wildtype mice. Injections were performed prior to training in the Morris water maze and for contextual fear conditioning. Both paradigms are hippocampus-dependent learning and memory paradigms; Morris water maze tests spatial navigation learning and memory while contextual fear conditioning tests associative learning and memory. In both tasks, mice receiving 200 pM $A\beta_{1-42}$ showed improved performance during the testing phase; neither wildtype receiving scrambled peptide nor α7 nAChR null mice receiving $A\beta_{1-42}$ showed signs of cognitive enhancement. Collectively, these findings suggest that $A\beta_{1-42}$ may be an endogenous neuromodulatory peptide that, at least in hippocampus, utilizes α7 nAChRs to exert its effects.

A second publication from Puzzo et al. tackled this ambitious hypothesis [37]. First, it was demonstrated that hippocampal (but not cerebellum) $A\beta_{x-42}$ level increased following theta-burst stimulation of Schaffer collaterals and training for contextual fear memory. The next experiment reduced endogenous rodent Aβ through antibody depletion and siRNA methodologies prior to hippocampal LTP induction or training for contextual fear memory. The resultant inhibition of LTP and contextual learning lends further support to the model developed from the set of studies described above. Specifically, antirodent Aβ antibodies and siRNA against rodent APP inhibited Schaffer collateral–CA1 LTP as well as contextual fear memory; exogenous application of human $A\beta_{1-42}$ oligomers (but not monomers) restored proper function. Furthermore, Aβ depletion strategies that diminished both post-tetanic potentiation (due to enhance neurotransmitter release) and LTP in wildtype hippocampal slices were unsuccessful in slices obtained from α7 nAChR knockout mice. Thus, it appears that endogenously produced oligomeric Aβ is capable of supporting hippocampal synaptic plasticity, learning and memory and utilizes α7 nAChRs.

From these sets of studies, Aβ interaction with α7 nAChRs clearly has both positive and negative effects on neurotransmitter release, synaptic transmission, synaptic plasticity, learning and memory. What decides one outcome over the other? The simplest answer to this question is Aβ concentration in that picomolar concentrations of focally (acutely) delivered Aβ potentiates glutamatergic neurotransmission, synaptic potentiation, and enhanced learning and memory. High concentrations (nanomolar-μM) of acutely applied Aβ led to reduced synaptic NMDA receptors, reduced glutamatergic transmission and, presumably, impaired synaptic potentiation. Alternatively, Aβ-induced LTP impairment and cognitive deficits can be achieved with moderate concentrations of Aβ when it is delivered for an extended time period (300 pmoles/day). Thus, as is the case with traditional nAChR agonists, acute exposure to moderate doses leads to receptor activation; exposure to high concentrations or prolonged exposure to moderate concentrations can lead to receptor inactivation through a desensitization mechanism.

5.3. Glial α7 nAChRs

An emerging issue in the field of α7 nicotinic receptor research is to decipher the functional role of these receptors on glial cells. Glial cells outnumber neurons in the brain and their traditional designation as housekeeping cells continues to be reconsidered as experimental observations indicate direct glial contributions synaptic function. Glial cells express functional receptors to many neurotransmitters and neuromodulators including, glutamate, GABA, ATP and ACh and there is now evidence of α7 nAChRs expression on most of the major glial types: microglia, NG2 cells and astrocytes [97–100]. The activation of α7 nAChR on cultured microglia reduces the release of a major inflammatory mediator in the CNS, TNF-α [87]. Given that TNF-α is abundantly found in the AD brain suggests that, under AD-like excess Aβ conditions, these receptors may be chronically inactivated through prolonged interaction with Aβ. Brain microglia and therefore brain inflammation are subject to α7 nAChR regulation which has direct relevance to AD.

Because astrocytes can release neurotransmitters to modulate neuronal excitability and synaptic transmission, there is much interest in the role of astrocytes in brain function. Since α7 nAChRs flux Ca^{2+}, and changes in intracellular Ca^{2+} are the basis of astrocytic "excitability", the activation of these receptors could be a potent mechanism for modulating astrocytic activity [88]. In cultured hippocampal astrocytes Sharma and Vijayaraghavan [86] found that focal ACh application induced inward currents in recorded astrocytes which were blocked by MLA. Ca^{2+} imaging experiments revealed intracellular Ca^{2+} elevations that persisted for tens of seconds, which were shown to be caused by an extracellular Ca^{2+} influx eliciting ryanodine receptor mediated CICR.

Evidence of α7 nAChR-mediated astrocyte functional responses in slice preparations is scarce. However, we have shown astrocytic Ca^{2+} elevations in slice preparations of hippocampus and neocortex elicited by focal nicotine and $A\beta_{1-42}$ (100 pM) application; these experiments were performed in the presence of tetrodotoxin (TTX) suggesting that local network activity was not responsible for the observed astrocytic Ca^{2+} elevations [142]. The α7 nAChRs competitive antagonist, MLA, blocked these responses.

Additional evidence for functional α7 nAChRs on glial cells comes from α7 nAChR-mediated inward currents in area CA1 NG2 cells from hippocampal slices in response to nicotine and [101]. These currents were potentiated by the potent α7 nAChR allosteric modulator PNU-120596 and blocked by the α7 nAChR-selective antagonist MLA, but not DHβE, an α4 nAChR specific antagonist. These studies have significance because NG2 cells have been postulated to be glial precursors; therefore, Aβ–α7 nAChR interaction may differentially lead microglial versus astrocyte genesis.

In summary, evidence is emerging that glial-resident α7 nAChRs can functionally contribute to NG2 cell development, modulate TNF-α production by microglia, and induce intracellular Ca^{2+} signaling in astrocytes that impinge upon neuronal synaptic signaling. In some cases, it is evident that Aβ modulates these responses therefore implicating the Aβ–α7 nAChR interaction on glia in AD inflammation and possibly cognitive function.

6. nAChRs, Aβ, and Alzheimer's disease

Understanding the molecular mechanism behind the selective vulnerability of cholinergic neurons to Aβ toxicity would greatly advance our capabilities in treating AD. The fact that vulnerable neuron populations happen to be enriched for α7 nAChRs may provide an important clue. As discussed previously, one possibility as Aβ accumulates during AD is that the neuroprotective function of nAChR activation is blocked by the antagonizing effect of Aβ peptides. Another possibility is that the Aβ–nAChR interaction under disease conditions directly contributes to neurotoxicity.

Potential mechanisms for each of these possibilities will be discussed in the following sections.

6.1. nAChRs protect against Aβ toxicity

In vitro studies utilizing cultured neurons have demonstrated that α7 nAChRs mediate, at least in part, the neuroprotective effects of nicotine against Aβ toxicity [102]. Protection against Aβ toxicity is proportional to the number of α7 nAChRs expressed by cultured cells [103]. Chronic exposure to $A\beta_{1-42}$ *in vitro* leads to up-regulation of α7 nAChRs in a manner similar to the effects of chronic nicotine treatment [50,78]. Tg2576 mice that produce excessive Aβ continue to up-regulate cortical and hippocampal α7 nAChRs as these animals age possibly providing an explanation as to why this AD model does not exhibit a cholinergic lesion phenotype nor significant loss of hippocampal and neocortical neurons [50,78,104–107].

α7 nAChR-mediated neuroprotection against Aβ is via activation of the PI3K pathway; several lines of evidence suggest that this can occur through transactivation of src and tyrosine kinase receptors, including the high-affinity NGF receptor, TrkA [80,108–111]. Paradoxically, at low to moderate concentrations of soluble $A\beta_{1-42}$, PI3K is also activated, suggesting that when Aβ is soluble and at non-disease concentration, the Aβ–nAChR interaction can lead to activation of neurotrophic mechanisms [38]. *In vitro* and *in vivo*, chronic nicotine leads to an increase in TrkA, in addition to α7 and α4β2 nAChRs; *in vivo*, this is accompanied by up-regulation of ChAT and VAChT in hippocampus [111–113]. Increased TrkA is neuroprotective against Aβ toxicity; high concentrations of Aβ are neurotoxic and block nicotine-induced TrkA up-regulation [110,111]. Thus, nAChRs are neuroprotective both by modulating the neurotrophic system crucial for the maintenance of cholinergic neuron integrity as well as stimulating signal transduction pathways that support neuron survival. Additionally, these studies suggest that in a situation of excess Aβ, nAChR function is blocked thus blocking its trophic activity and possibly contributing to Aβ toxicity. Taken together, one might imagine that under normal physiologic conditions, an Aβ–nAChR interaction provides a trophic signal; as Aβ accumulates, this interaction either blocks nAChR-mediated trophism or the Aβ–nAChR interaction under these circumstances becomes toxic.

6.2. Multiple opportunities for an α7 nAChR–Aβ interaction to contribute to AD etiology

Estimates of Aβ content in non-demented brain report picomolar values, however these estimates increase to nanomolar quantities for AD brain [46,47,83]. Several studies report that prolonged exposure of nAChRs to nanomolar Aβ results in significant block of receptor function [54–58,68]. This suggests that under disease conditions an Aβ–nAChR interaction would interfere with the normal function of these receptors. Given the overwhelming evidence that nAChRs perform a neuroprotective role, an Aβ–nAChR interaction under elevated Aβ conditions may exacerbate the toxicity of Aβ by diminishing the neuroprotective signaling performed by these receptors. The current literature indicates that additional outcomes of an Aβ–nAChR interaction under 'high Aβ' conditions could yield (1) perturbation and dysregulation of signal transduction mechanisms involved in synaptic plasticity and homeostasis; (2) receptor–peptide complex internalization; (3) cell toxicity; and (4) plaque seeding. The evidence for these mechanisms will be discussed in the following section.

While there is a general consensus that the presence of excess Aβ is perhaps the most fundamental neurotoxic event in AD, several lines of evidence indicate that oligomeric, soluble forms of Aβ, rather than amyloid plaques, initiate the cognitive deficits characteristic of the disease [114,115]. For example, transgenic mouse models for AD in which Aβ is over produced and accumulates in the CNS develop memory impairments long before plaques are detected and in the absence of significant neuronal loss [78,104,105,116]. Furthermore, introduction of Aβ oligomers produced *in vitro* or *in vivo* induces learning and memory deficits in wildtype rodents that resemble those of transgenic models for AD [33,34,117]. Therefore, some of the cognitive impairments in AD may not be associated with extensive neuronal death; rather, they may be the result of more subtle functional changes induced by soluble Aβ. It will be important for future studies of the Aβ–nAChR interaction to attribute outcomes of this interaction not only to the concentration of soluble Aβ but also to specific structures and aggregates of the peptide.

In addition to extracellular deposits of insoluble Aβ in plaques that are a primary histopathological diagnostic marker for AD, observations made as the 20th century yielded to the 21st, identified Aβ immunostaining within neurons and glia of post-mortem AD samples [118,119]. Later, it was discovered that α7 nAChRs are not only expressed on neurons, this receptor is expressed by astrocytes and microglia [86,87]. Consequently, several groups have investigated the possibility that an α7 nAChR–Aβ interaction on these cell types are part of AD etiology.

In a series of publications from Wang, Nagale, D'Andrea and colleagues, this group first explored the model that an α7 nAChR–Aβ interaction leads to intracellular accumulation of Aβ. Initial work utilizing post-mortem AD brains and immunostaining approaches revealed that $A\beta_{1-42}$ was localized intracellularly in neurons and astrocytes of AD brains; neurons and astrocytes that had accumulated large amounts of $A\beta_{1-42}$ also highly expressed α7 nAChRs [41,120–123]. In neuroblastoma cells transfected with α7 nAChR cDNA, transfected cells exhibited rapid binding, internalization and accumulation of exogenous $A\beta_{1-42}$, but not $A\beta_{1-40}$; this internalization was related to the level of α7 nAChR expression [41]. Further, the α7 nAChR antagonist, BTX, prevented $A\beta_{1-42}$ uptake. These results suggest that α7 nAChRs facilitate $A\beta_{1-42}$ internalization and may confer selective vulnerability of specific cell types to the toxic effects of intracellular $A\beta_{1-42}$. Nagale and colleagues took this notion a step further by suggesting that α7 nAChR–Aβ interaction and internalization may actually lead to plaque formation when the host cell eventually dies and deposits the intracellular contents in the brain parenchyma [121,122,124].

The identification of α7 nAChR and Aβ within astrocytes also provided the first indication that nAChR–Aβ interaction may be an important event in the inflammatory progression of the disease [121]. These studies showed that $A\beta_{1-42}$ and α7 nAChR proteins were co-localized in intensely GFAP-positive (activated) astrocytes in immunostained AD brain. Since these studies also identified ChAT, the authors proposed a model in which Aβ and α7 proteins are phagocytosed by activated astrocytes in the vicinity of neuronal remnants. As neuronal debris accumulates in the astrocyte, astrocyte viability is compromised and eventually kills the cell leaving behind Aβ deposits rich in astrocytic GFAP, and neuronal markers such as ChAT and α7 protein.

A slightly different interpretation was made by Teaktong et al. [125] when they found that the majority of astrocytes in AD hippocampus and cortex also *express* α7 nAChRs; this group deduced that α7 nAChRs are up-regulated on astrocytes in AD. Follow-up studies determined that the number of astrocytes double-labeled with α7 nAChR and GFAP antibodies was increased in most areas of the hippocampus and entorhinal cortex in AD compared with controls suggesting that increased astrocyte alpha7 nAChRs in AD may be associated with inflammatory mechanisms related to degenerative processes [126].

Although the work by Teaktong et al. and Nagale et al. utilized antibodies to α7 nAChRs that have since come into question as to

their specificity, subsequent work from Dr. Agneta Nordberg's group using ^{125}I-BTX to quantify α7 nAChR protein supports these initial observations both in post-mortem AD brain and primary cell culture [127–129]. Subsequent *in vitro* work by Xiu et al. [129] lends support to the idea that Aβ-induced α7 nAChR up-regulation on astrocytes occurs in the disease: exposure of cultured primary astrocytes to picomolar–nanomolar $A\beta_{1-42}$ for 48 h followed by quantification of mRNA and protein with RT-PCR and immunoblot, respectively, resulted in up-regulation of both α7 nAChR mRNA and protein.

A recent study utilizing cultured rat microglia found that activation of microglial α7 nAChR leads to increase Aβ phagocytosis [130]. The study found that human $A\beta_{1-42}$ clearance was increased by nicotine as well as the cholinesterase inhibitor and α7 nAChR allosteric modulator galantamine; Aβ clearance was blocked by the broad nAChR antagonist MEC and the α7 nAChR-selective antagonist MLA, but not atropine a mAChR antagonist. Furthermore, galantamine-treated AD mice exhibited reduced amyloid load as did rats that received intra-hippocampal injections of human $A\beta_{1-42}$. Thus, these studies suggest that microglia-resident α7 nAChRs may be part of the mechanism mediating the therapeutic efficacy of this compound through Aβ clearance.

The interaction of α7 nAChRs on astrocytes with Aβ peptide may provide a possible link between α7 nAChRs and the inflammatory processes of AD. Analogous to its role in the periphery, α7 nAChR activation on glia shunts TNF-α production and release; therefore, an important question to answer is under what conditions might an α7 nAChR–Aβ interaction lead to decreased TNF-α production as opposed to general microglial and astrocyte activation. It will be important to decipher this apparently complex relationship between neuroprotection, astrocyte activation, inflammation, and neurotoxicity, in addition to how this interaction contributes to the development of neuronal- and astrocyte-derived plaques in AD brain. Clearly, an interaction between glial-resident α7 nAChRs and Aβ may be involved in a broad array of outcomes during the progression of AD.

We recently tested the hypothesis that α7 nAChRs are neuroprotective during early stage AD by investigating the effects of α7 nAChR gene deletion on cognitive function and septo-hippocampal integrity in the Tg2576 APP transgenic animal model for AD [131]. Whereas α7 nAChR knockout (A7KO) mice neither show cognitive deficits nor exhibit morphological CNS abnormalities, we found that cognitive deficits seen in 5-months-old APP transgenic mice are more severe when α7 nAChR receptors are absent (A7KO-APP) [93,131,132]. Biochemical analyses on 5-months-old A7KO-APP revealed significant reduction in hippocampal and basal forebrain ChAT activity and loss of hippocampal neurons and markers compared to APP mice. Consistent with lesion studies and observations in AD brain, compromise of basal forebrain cholinergic function leads to similar concessions within the hippocampus of 5-months-old A7KO-APP mice. These studies demonstrated that α7 nAChRs mediate neuroprotective mechanisms that maintain the basal forebrain cholinergic phenotype and preserve hippocampal integrity; loss of basal forebrain cholinergic integrity is accelerated and exacerbated when α7 nAChRs are absent and misfolded Aβ is in excess.

Data continues to accumulate demonstrating that Aβ peptides interact with α7 nAChRs with especially high-affinity and for extended periods of time; consistent with such an interaction, up-regulation of α7 nAChRs mRNA and protein has been reported in astrocytes, peripheral blood leukocytes and cortical and hippocampal neurons harvested from the tissue of AD patients [10,125,133]. The additional observation that, in early AD, Aβ preferentially accumulates in neuronal populations that are enriched for α7 nAChRs may be one reason for the selective vulnerability of the basal forebrain cholinergic system to Aβ toxicity.

In one study, mRNA expression levels of nicotinic and muscarinic AChR subtypes and ChAT were measured in single cells isolated from the cholinergic basal forebrain of post-mortem AD tissue (and non-cognitively impaired controls) then individually analyzed using microarray methods. No differences in mRNA expression were observed for the other nAChR subunits, mAChR subtypes or ChAT [16]. However, cells from AD basal forebrain exhibited a significant up-regulation of α7 nAChR subunit mRNAs [16]. This increase in α7 nAChR expression levels within CBF neurons was inversely correlated with Global Cognitive Score and with Mini-Mental State Examination performance [16]. We would posit that these increases in α7 nAChR protein within the basal forebrain result from direct interaction with Aβ and receptor desensitization followed by receptor up-regulation [66]. In fact, α7 nAChR-selective agonists are unable to activate these receptors in APP transgenic mice and recent work on human AD post-mortem brain samples indicate that much of the receptor protein is functionally inactivated due to association with Aβ peptide [9,10,134,135]. In addition, recent studies have shown that these Aβ–α7 nAChR protein complexes occur primarily in brain regions targeted by the cholinergic basal forebrain; disruption of this association in post-mortem AD cortex leads to increased availability of functional α7 nAChRs [10]. These observations suggest that in AD, α7 nAChRs are likely inactive due to desensitization as a consequence of prolonged association with Aβ peptide.

Based on current understanding, we propose that soluble Aβ oligomers that may lead to the transient activation of α7 nAChRs and subsequent initiation of both neuroprotective and neurotrophic signaling mechanisms that have been elucidated *in vitro* [38,50,80]. An additional benefit may be provided via sequestering Aβ oligomers and preventing further oligomerization, thus deviating Aβ from additional toxic interactions (e.g., mediators of glutamatergic neurotransmission); [136,137]. As AD progresses, we envision that Aβ accumulates and irreversibly associates in a manner that overwhelms the availability of α7 nAChRs leading to functional blockade and loss of neuroprotective signaling.

6.3. Therapeutic opportunities

While much progress has been made regarding the nature of α7 nAChR–Aβ interaction *in vivo* and *in vitro*, many questions remain as to the exact features of α7 nAChR–Aβ interaction during the initiation and progression of AD to confidently suggest viable therapeutic strategies. Nonetheless, one might consider a few possibilities based on the extant literature reviewed here. Several α7 nAChR agonists and positive allosteric modulators (PAMs) have been developed as therapeutic agents targeting central and peripheral disorders that involve pain, inflammation, schizophrenia, and AD [138–140]. One such compound, S-24795, was recently directly tested for efficacy in AD; application to homogenates prepared from post-mortem AD brain was found to facilitate Aβ dissociation from the receptor in order to resurrect α7 nAChR function and its neuroprotective properties [10,42]. Possibly, S-24795, and other such α7 nAChR PAMs would prove beneficial during early AD by both inhibiting and partially reversing Aβ binding to α7 nAChRs. However it remains to be seen if the dislodged Aβ is then free to interact in alternative yet deleterious ways. Possibly coincident Aβ immunotherapy would alleviate this potential negative side effect of α7 nAChR PAM therapy. Another possible strategy, albeit somewhat difficult to envision at the receptor level, would be to develop a compound that is capable of maintaining α7 nAChR neuroprotective signaling capabilities on the one hand and continue to sequester Aβ on the other. Again, this in conjunction with interventions that decrease oligomeric Aβ levels might prove most efficacious.

Assuming that, under normal physiological conditions, Aβ and α7 nAChRs interact and result in receptor activation implies that

this interaction may serve a neuroprotective role given that α7 nAChRs couple to neuroprotective signaling cascades (PI3K etc.). Therefore, it seems imprudent to prophylatically block *all* α7 nAChR–Aβ interaction. However, as AD progresses and soluble Aβ acquires pathological concentrations and conformations, it might be useful to develop ways in which to interrupt specific α7 nAChR–Aβ interactions, especially if this interaction antagonizes receptor function or is involved in accumulating intracellular Aβ. As is being currently pursued, targeting Aβ directly with immunotherapy is one appraoch.

From the nAChR side of the equation, the development of a decoy nAChR-like binding site (presuming it is known) could prevent toxic α7 nAChR–Aβ interactions. Direct modulation of nAChR function is another strategy. However this requires a solid understanding of the functional relationship between the receptor and peptide as Aβ levels increase and different conformations of the peptide accumulate with disease progression. Clearly, while great strides have been made in understanding the α7 nAChR–Aβ interaction in recent years, the likely complex nature of this relationship as AD progresses and soluble Aβ acquires additional aggregation conformers demands that much has yet to be understood before emphatically stating what the best α7 nAChR therapeutic strategy is for AD.

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