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ABSTRACT

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Alzheimer's disease (AD) is a neurodegenerative disorder that manifests in the form of deficiencies in cognitive processes such as memory and learning. The pathological features of AD include hyperphosphorylated tau proteins that form neurofibrillary tangles as well as senile plaques composed primarily of the peptide β-amyloid (Aβ). When present in high concentrations in the brain, Aβ inhibits certain subtypes of neuronal nicotinic acetylcholine receptors (nAChRs) in the hippocampus. The effects of Aβ in the hippocampus have proven to be neurotoxic, resulting in reduced functionality of nAChRs and the subsequent death of neurons in the cholinergic pathway. The early stages of AD are characterized by reduction of nAChR density and by degeneration of the cholinergic neurons that provide input to the hippocampus. Because the hippocampus plays a critical role in memory formation and other cognitive processes, dysfunction in this brain region results in significant cognitive deficiencies. Understanding the interaction between Aβ and the structurally and functionally diverse nAChR subtypes and possible downstream effects in signaling cascades that might result from that interaction are important steps in comprehending AD pathogenesis. Comprehension of this interaction and factors that might influence it could lead to the development of pharmaceutical agents useful in the treatment of AD.

Keywords: Alzheimer's disease, nAChR, beta-amyloid, Congo Red, antibiotics

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INTRODUCTION

Alzheimer's Disease (AD)

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and other cognitive deficiencies. It is the most common cause of dementia affecting nearly 25 million people worldwide (Conejero-Goldberg, Davies et al. 2008). By 2050 it is expected to affect 13.2 million individuals in the United States alone (Buckingham, Jones et al. 2009).

Although considerable progress in understanding the etiology and pathology of AD has been attained, the mechanisms underlying the pathogenesis of the disorder remain somewhat obscure (Tang, An et al. 2008). However, several pathological features of the disease have been identified. The two major neuropathological hallmarks of AD are deposition of beta-amyloid (Aβ) plaques and neurofibrillary tangles in various brain regions. In the early stages of AD, these observations are accompanied by selective cholinergic denervation and decreased nicotinic acetylcholine receptor (nAChR) density (Arias, Alés et al. 2004; Ellis, Villemagne et al. 2008; Barrantes, Borroni et al. 2010; Hernandez, Kayed et al. 2010). Studies, both *in vivo* and post mortem, in AD patients indicate cholinergic neuron death as well as a significant reduction in the amount of nAChRs with some subtypes affected more than others. The deficits in learning and memory associated with AD progression reflect this incremental loss of cholinergic neurons and nAChR function/expression in certain brain regions (Wevers, Burghaus et al. 2000; Barrantes, Borroni et al. 2010). In other words, there is a correlation between cholinergic degeneration, nAChR destruction and AD severity (Pettit, Shao et al. 2001; Ellis, Villemagne et al. 2008). Currently, there are no treatments for this neuronal loss.

The neuronal loss characteristic of AD progression selectively affects certain regions of the brain more than others. In studying AD, the brain structure known as the hippocampus is of particular interest due to its role in various cognitive functions. The hippocampus, which is part of the limbic system, is located in the medial temporal lobe of the brain and plays important roles in the consolidation of information from short-term memory to long-term memory as well as spatial navigation and long-term potentiation. Not surprisingly, the selective cholinergic denervation seems to be most severe in the temporal lobes and adjacent limbic and paralimbic areas (Parri, Hernandez et al. 2011). The hippocampus is an early target of the disease-related denervation with the progression of the disease leading to destruction of neurons in the entorhinal cortex and eventually, the neocortex (Buckingham, Jones et al. 2009; Ikonomovic, Wecker et al. 2009; Parri, Hernandez et al. 2011).

The pathogenesis of AD as it is observed in the hippocampus is in large part related to the neuronal organization in the hippocampus as well as the distribution of nAChR subtypes therein. Structurally, the hippocampus is composed of two regions, the dentate gyrus (DG) and the Cornu Ammonis (CA). Each of these regions is composed of a main cell layer, the principal cell layer. The nerve cells of the principal cell layer of the DG are the granule cells and of the CA regions, the pyramidal cells. The primary cholinergic input to the hippocampus originates in the basal forebrain, specifically the medial septum-diagonal band complex (MSDB), and innervates inhibitory GABAergic interneurons found within the hippocampus (Albuquerque, Pereira et al. 1998; Sudweeks and Yakel 2000; Parri, Hernandez et al. 2011). Incidentally, post mortem studies have shown that the most vulnerable neurons in patients with AD are the basal forebrain cholinergic neurons (Barrantes, Borroni et al. 2010).

The cholinergic destruction in the hippocampus and resultant cognitive dysfunction seem to be related to nAChR subtype distribution within its neuronal populations. Neuronal populations within the hippocampus have been shown to express multiple nAChR subtypes (Gotti, Zoli et al. 2006). The nAChRs are expressed both presynaptically on the cholinergic neurons where they influence neurotransmitter release, and postsynaptically on the somatodendritic region of GABAergic interneurons on which the cholinergic neurons synapse (Pettit, Shao et al. 2001; Gotti, Moretti et al. 2006; Zolles, Wagner et al. 2009). In normal cognitive processes, these hippocampal nAChRs, in conjunction with cholinergic innervation, modulate GABA transmission and coordinate the synchrous activation of pyramidal cells populations (Albuquerque, Pereira et al. 1997; Gotti, Zoli et al. 2006).

Multiple studies have shown high densities of α7 and α4β2 nAChRs in cholinergic neurons and interneurons in the hippocampus (Hellstrom-Lindahl, Mousavi et al. 1999; Gotti, Zoli et al. 2006). In fact, the most vulnerable neurons targeted for destruction in AD are those expressing high levels of α7 nAChR (Barrantes, Borroni et al. 2010). Because they are commonly recognized as the primary nAChR subtype in the hippocampus, α7 nAChRs have been studied extensively and a small part of their role in the etiology of AD has been elucidated. However, there is still much to be learned concerning the interaction between these α7 subtype receptor and the Aβ peptide.

Nicotinic Acetylcholine Receptors (nAChRs)

Nicotinic acetylcholine receptors (nAChRs) comprise a class of cys-loop, ligand-gated cationic channels characterized by their ability to be activated by both the endogenous neurotransmitter acetylcholine and the exogenous nicotine molecule (Hogg, Raggenbass et al.

2003; Dajas-Bailador and Wonnacott 2004; Gotti, Zoli et al. 2006). The structure of the channel consists of five protein subunits arranged around a centralized cation-permeable pore that opens in response to the binding of a ligand (Buckingham, Jones et al. 2009; Barrantes, Borroni et al. 2010). The twelve different subunits (α2-10, β2-4) identified in mammalian brains can be combined as homo-pentameric (e.g. five α7 subunits) or as hetero-pentameric (e.g. two α4 and three β2 subunits) receptor complexes with each subtype exhibiting distinct pharmacological and biophysical properties (Gotti and Clementi 2004; Zolles, Wagner et al. 2009). Of the neuronal nAChRs subtypes, the homomeric α7 and heteromeric α4β2 and α3β4 subtypes have been shown to be the most widely expressed in the brain (Buckingham, Jones et al. 2009). For this reason, these subtypes have been extensively characterized, relative to other subtypes.

The variability found in nAChR subtypes accounts for their physiological versatility in the body, as nAChRs are widely distributed throughout both the CNS and PNS where they participate in a variety of physiological processes (Gotti and Clementi 2004). In the CNS, the coordination between nAChRs and acetylcholine-producing cholinergic neurons plays a critical role in the modulation of processes such as neurotransmitter release, cell excitability and neuronal integration. Each of these processes influences higher cognitive processes like learning, memory and attention (Pettit, Shao et al. 2001; Gotti, Zoli et al. 2006). While proper functioning of neuronal nAChRs results in the ability to perform cognitive functions and protection from neurodegenerative diseases, cholinergic and receptor dysfunction can contribute to a number of pathologies, including Parkinson's, epilepsy and Alzheimer's disease (Nordberg 2001).

The cation conductance characteristic of nAChRs has more significant implications than simply membrane depolarization. Activated α7 nAChRs are involved in a number of intracellular events via local calcium signaling that initiates second messenger systems (Charpantier, Wiesner et al. 2005). Their participation in a variety of signaling pathways might explain the duality of roles of the receptor as it has been implicated in both neuroprotection and neurotoxicity (Buckingham, Jones et al. 2009). The fate of the cell depends on which pathway is activated, with certain pathways contributing to cell preservation, and others to cell death (Buckingham, Jones et al. 2009). The implications of pathway activation and the effect of Aβ and nAChRs on those pathways will be discussed in detail.

Beta-Amyloid (Aβ)

The role of nAChRs in the hippocampus-specific destruction is related in part to its interaction with Aβ (Liu, Gu et al. 2007). Aβ has been shown to be neurotoxic, and AD-like symptoms have been seen in mice transgenically overexpressing Aβ (Buckingham, Jones et al. 2009). Additionally, Aβ is an inhibitor of postsynaptic nAChRs in the rat hippocampus (Pettit, Shao et al. 2001).

The Aβ peptide is the product of amyloid precursor protein (APP) cleavage by β and γ-secretases, which results in Aβ peptides of varying lengths. An increased ratio of Aβ42 (42 amino acids long) to Aβ40 (40 amino acids long) is associated with AD (Kumar-Singh, Theuns et al. 2006). Individuals possessing genetic mutations in the genes that encode for APP and the APP-processing proteins, such as β and γ-secretases, demonstrate an increased likelihood for development of AD, which is likely attributable to increased production of the Aβ42 configuration (Hardy and Selkoe 2002; Glabe 2008).

Aβ42, in particular, is a very versatile peptide, possessing the ability to take on monomeric, oligomeric and fibrillar forms under normal physiological conditions (Luheshi, Tartaglia et al. 2007). The form of the amyloid depends upon a number of conditions including solution composition, length of the fragment, peptide concentration, age of the sample, presence of organic solvents, temperature, pH and ionic strength (Snyder, Ladror et al. 1994; Schmit, Ghosh et al. 2011). The highly aggregated amyloid fibril, which is characterized by extensive β-sheeting and hydrogen bonding, was at one time considered the most pathogenic form, but more recent studies implicate the soluble oligomeric configuration, citing a strong correlation between overall load of the soluble Aβ and cognitive decline (Dougherty, Wu et al. 2003; Walsh and Selkoe 2007). Many experimental designs reflect this paradigm shift in favor of the oligomeric Aβ42, which has become a popular target in studying the deleterious effects of the amyloid peptide. However, maintaining desired structure of the peptide in an experimental setting has proved difficult and is often times ignored when establishing experimental protocol. The unpredictability of the amyloid protein complicates the reproducibility of any experimental results derived from the use of the molecule. Not only can Aβ assume monomeric, oligomeric and fibrillar confirmations within solution, but it can also vary its structure significantly within those very generalized structural categories (Glabe 2008).

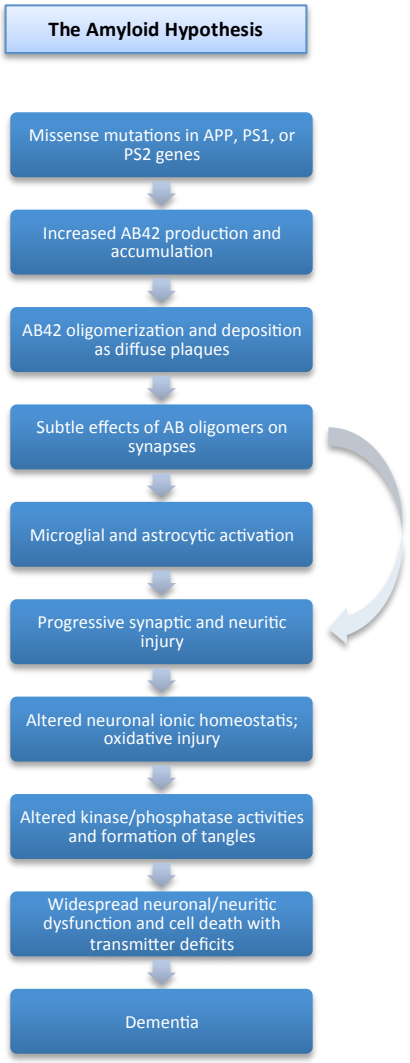


Figure 1: Theoretical cascade of events leading to AD (Hardy and Selkoe 2002).

The structural plasticity and resultant sensitivity to method of preparation and storage observed in Aβ is a possible explanation for the seemingly paradoxical conclusions that have been drawn up in the literature (Teplow 2006; Buckingham, Jones et al. 2009). It also emphasizes the importance of the standardized handling and storage of *in vitro* Aβ.

Beta-Amyloid/Nicotinic Acetylcholine Receptor Interaction

Because nAChRs are so functionally and structurally diverse, Aβ interacts differently with each subtype, making it difficult to predict the implications of those interactions. Some receptor subtypes are more prone to AD-related dysfunction than others. Due to the high density of α7, α4β2 and α3β2 nAChRs in the hippocampus, characterization of their interaction with Aβ is of particular importance. The effect of Aβ on the α7 subtype has been studied rather extensively, and Aβ has been shown to have high affinity for α7 nAChRs (Wang, Lee et al. 2000). In fact, the deposition of Aβ in the early stages of AD overlaps with α7 nAChR expression in the basal forebrain cholinergic system (Parri and Dineley 2010). Therefore, the neurons most prone to Aβ attack are those with large populations of a7 nAChRs (Li, Wu et al. 2011).

As mentioned before, the nAChR activation is likely upstream to both pathogenic and protective events (Buckingham, Jones et al. 2009; Wang, Stucky et al. 2009). The relationship between second messenger systems, Aβ, nAChRs and downstream events is quite convoluted but important to investigate in order to understand the complexity of the Aβ/nAChR interaction in the disease state.

One of the pathways linked to the neuroprotective role of nAChRs is the phosphatidylinositol 3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/AKT) pathway, a pathway linked to anti-apoptotic cellular activities (Kihara, Shimohama et al. 2001).

Activation of the nAChR by nicotine has been shown to increase phosphorylation of AKT as well as the expression and phosphorylation of B-cell chronic lymphocytic leukemia/lymphoma (BCL2), a downstream component of the PI3K/AKT and known antiapoptotic protein (Kihara, Shimohama et al. 2001; Arias, Alés et al. 2004). The neuroprotective effects of this cascade were inhibited in the presence of nAChR antagonists (Buckingham, Jones et al. 2009). In examining the connection between nAChRs and the PI3K/AKT pathway, a tyrosine kinase known as FYN has been identified as a possible contributor to the nAChR/PI3K relationship. Not only has FYN, a member of the Src-family of kinases (SFKs), been shown to be involved in phosphorylation events in the PI3K pathway, including the phosphorylation of AKT, but it was also shown to be attached to both the nAChR and a subunit of PI3K in fetal rat cortical cell cultures (Kihara, Shimohama et al. 2001; Tang, Feng et al. 2007). Additionally, the Src-family of kinases has been shown to directly interact with the cytoplasmic loop of α7 nAChRs (Charpantier, Wiesner et al. 2005).

Just what role FYN plays in the apoptosis/antiapoptosis scheme is unclear as different studies report very different findings. Mutant α7 nAChRs lacking cytoplasmic loop tyrosine residues were more active than wild-type receptors and insensitive to kinase or phosphatase inhibition. Tyrosine kinase inhibition by genistein decreased α7 nAChR phosphorylation and strongly increased acetylcholine-evoked currents, whereas tyrosine phosphatase inhibition by pervanadate produced the opposite effect (Charpantier, Wiesner et al. 2005). Whether or not this phosphorylation/dephosphorylation dynamic results in increased surface receptors is disputed. However, the evidence seems to suggest that the changes in nAChR responsiveness are not due to increased/decreased receptor number, but rather to conformational changes in

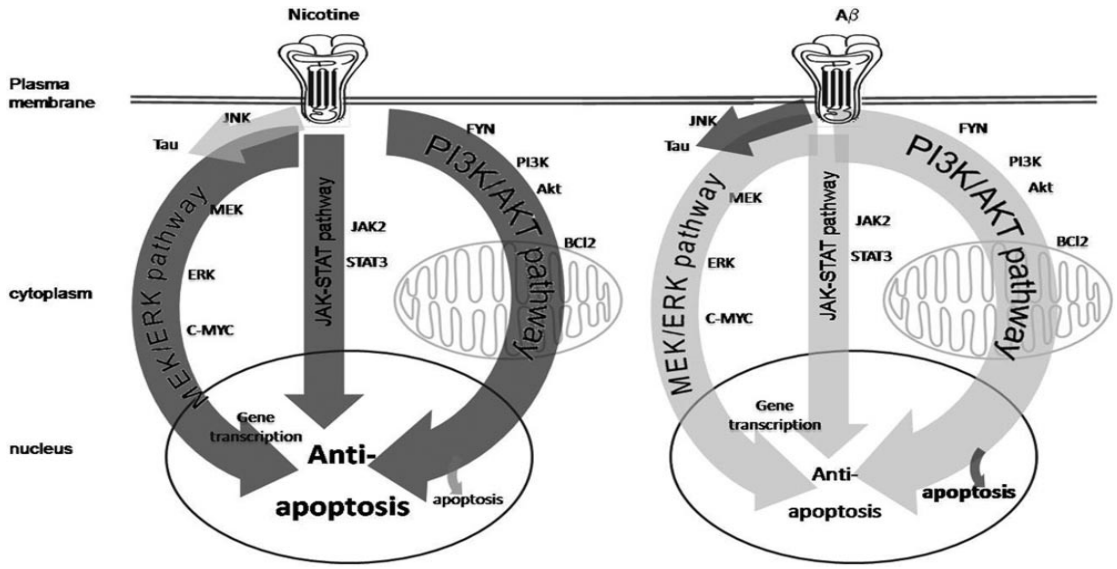


Figure 2: Model of the various pathways activated by nAChR agonists, nicotine or Aβ and how those pathways are involved in determining cell fate (Buckingham, Jones et al. 2009).

the receptor as a result of FYN activity (Charpantier, Wiesner et al. 2005; Cho, Song et al. 2005). But what seems to remain a consistent assertion is the idea that the phosphorylation state of the receptor has implications in the functionality and survivability of the cell.

The actions of FYN are significant because, while it appears to play a neuroprotective role under normal physiological conditions, it has also been shown to be involved in cell dysfunction when activated by Aβ, an idea supported by the finding that Aβ42 induces tau protein phosphorylation through its interaction with α7 nAChRs and FYN (Wang, Li et al. 2003; Buckingham, Jones et al. 2009). The idea of a toxic relationship between Aβ and nAChRs was further confirmed by the finding that Aβ-induced lethality in SH-SY5Y cells was reduced when α7 was rendered inert by RNAi (Buckingham, Jones et al. 2009). Aside from abnormal activation of FYN, the neurotoxic effects of Aβ activation of nAChRs have been linked to the MAPK/ERK-2 and MAPK/JNK pathways, which are also involved in tau hyperphosphorylation and apoptotic processes (Buckingham, Jones et al. 2009). Which pathway is activated by Aβ and the pathway's

effect on cell viability is dependent upon the concentration of Aβ as well as the length of Aβ exposure (Bell, O'Riordan et al. 2004; Buckingham, Jones et al. 2009).

The nicotinic acetylcholine receptor's participation in these second messenger systems is important as it introduces yet another mode whereby the receptor can interact with molecules like Aβ. Because nAChRs are known participate in cognitive processes in the hippocampus and have been implicated in AD pathogenesis due to their interaction with Aβ, nAChRs have become targets of pharmacological intervention in the treatment of AD (Wevers, Burghaus et al. 2000; Pettit, Shao et al. 2001).

Congo Red

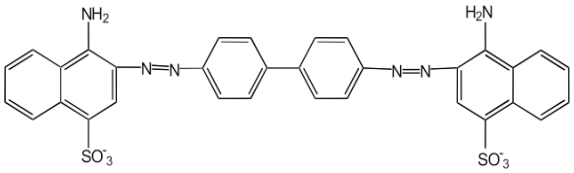


Figure 3: Chemical structure of Congo Red (Nilsson 2004).

Just how Aβ interacts with nAChRs is largely dependent on the amyloid structure. Changes in that structure can drastically affect experimental results. Establishing amyloid structure is of the utmost importance in standardizing experimental results. There are several accepted and common methods for detecting and quantifying the level of Aβ fibrillation *in vitro*, including circular dichroism spectroscopy, Thioflavin T (ThT) fluorescence, Congo Red binding, transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR) (Nilsson 2004). One of those methods, the Congo Red assay, has proved to be a cheap and effective method for quantifying aggregation due to the availability of the reagents. Congo Red, a secondary diazo dye, has been a commonly implemented tool for identifying amyloid fibrils in tissues post mortem (Nilsson 2004; Wu, Scott et al. 2012), but because of its ability to bind to the secondary β-sheet structure of the β-amyloid and the resultant change in absorbance spectrum of the dye,

Congo Red has been implemented as a means of identifying amyloid aggregation *in vitro* (Klunk, Jacob et al. 1999; Nilsson 2004). How the Congo Red binds to the amyloid fibrils is not exactly known, but with newer imaging techniques, the interaction between the two molecules is slowly becoming clearer. A series of intermolecular forces and binding sites are likely to participate in the CR/Aβ fibril interactions, including hydrogen bonding, ionic interactions, hydrophobic interactions and steric relationships (Nilsson 2004). When bound to amyloid fibrils, the CR molecules lie parallel to the fibril axis, resulting in torsional constriction and a shift in UV absorbance (Wu, Scott et al. 2012). The resultant shift in in absorbance maximum, from 490 nm to 540 nm, observed when amyloid samples are added to CR can be translated into concentration of CR bound to Aβ through a series of mathematical calculations. Calculating the amount of bound CR by observing maximal spectral shift at 540 nm, allows for the relative quantification of amyloid fibrils. Because the red shift in absorption maximum is only seen when CR binds to amyloid fibrils and not the soluble monomeric or oligomeric isoforms, an increase in CR bound to Aβ indicates an increase in fribrillation (Klunk, Jacob et al. 1999). Lack of shift in spectral absorbance indicates the absence of amyloid fibrils.

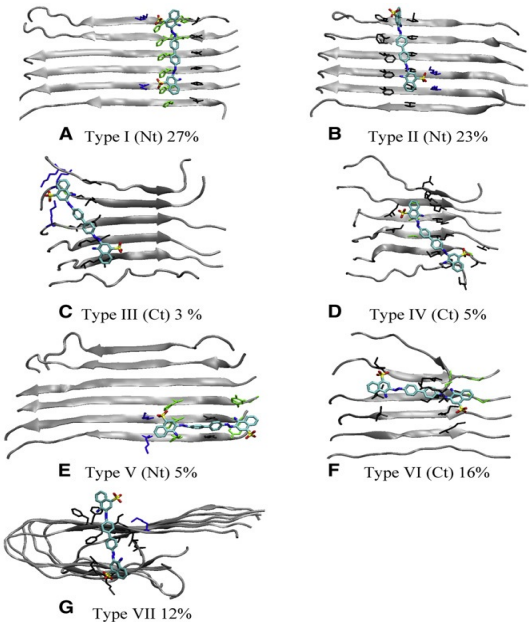


Figure 4: Seven proposed Aβ-CR binding sites (A-G) observed from binding simulations. Abundance is noted (Wu, Scott et al. 2012).

Antibiotics

Various factors affecting the nAChR/Aβ interaction have been discussed. Another potential factor that could play a role in the interaction is that of antibiotics. Antibiotics, specifically gentamicin and streptomycin, are commonly used in oocyte-incubating solutions as a means to maintain oocyte health. The use of antibiotics, like these, which are classified as aminoglycosides, has been linked to side effects such as ototoxicity, nephrotoxicity and neuromuscular block (Amici, Eusebi et al. 2005). It has been suggested that these side effects might be related to the interaction between aminoglycosidic antibiotics and nAChRs. Indeed, extended exposure to aminoglycosidic antibiotics such as gentamicin and streptomycin result in a decreased responsiveness to ACh by α7 nAChRs expressed in *Xenopus* oocytes, as evidenced by drops in peak response amplitude, as well as an increased susceptibility to desensitization. These effects were not easily reversible (Amici, Eusebi et al. 2005). A similar effect is seen in α9 nAChRs expressed in *Xenopus* oocytes when exposed to various antibiotics, including but not limited to aminoglycosides (Rothlin, Katz et al. 2000).

The reasons behind these effects are not well understood, but it has been suggested that aminoglycosides destabilize the open nAChR pore by blocking Ca^{2+} binding sites located in the extracellular region (Galzi, Bertrand et al. 1996; Rothlin, Katz et al. 2000; Amici, Eusebi et al. 2005). This theory is supported by the fact that increasing extracellular Ca^{2+} concentrations attenuates the inhibitory effects of the antibiotics (Rothlin, Katz et al. 2000). An alternate theory proposes that the decrease in response to ACh is voltage dependent and might be attributable to endogenous, Ca^{2+}-activated Cl^- channels (Amici, Eusebi et al. 2005). The fact that antibiotics affect the kinetics of α7 nAChRs so significantly complicates the matter even more as

it potentially adds another variable to the nAChR/Aβ equation. If both antibiotics and Aβ directly interact with the receptor, might their co-localization affect the Aβ/nAChR dynamic?

SPECIFIC AIMS

In this study we utilize human α7 nicotinic acetylcholine receptors expressed in *Xenopus laevis* oocytes to do the following:

1. Determine effect of Aβ on the kinetics of nAChRs.
2. Once the relationship between Aβ exposure and nAChR inhibition is established, examine the effect length of Aβ exposure has as it relates to the inhibition in ACh-activated nAChRs.
3. Examine different variables that might affect the interaction between Aβ and nAChRs, such as antibiotics and Aβ conformation.
4. Standardize our preparation of Aβ in order to enable accurate reproduction of experimental results by performing assays shown in the literature to effectively characterize and quantify Aβ form.

MATERIALS AND METHODS

Chemicals and Solutions

Oocyte Recording Solution

OR-2 solution with Ca^{2+} and theophylline (82 mM NaCl, 2.5 mM KCl, 1 mM Na_2HPO_4, 5 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ anhydrous, 500 mM theophylline anhydrous) was made by dissolving prepared salt solute in distilled water and adjusted to pH 7.5 with NaOH. OR-2 with Ca^{2+} and theophylline was supplemented with a combination of streptomycin (50 µg/mL) and penicillin (50 units/mL) (Invitrogen).

Beta-amyloid Preparation

Aβ42 and Aβ42 scramble (AnaSpec, Inc., Fremont, CA) were prepared from lyophilized powder stored at -20 degrees C and reconstituted in either distilled water or OR-2 with Ca^{2+} to a concentration of 1 mg/mL and briefly vortexed. The reconstituted peptide was diluted to a final stock concentration of either 10 µM, 1 µM, 500 nM or 100 nM and stored at 80 degrees Celsius. Immediately prior to administration of Aβ42, stock concentrations were thawed and diluted to 30 nM for use in experimentation. Unused stock aliquots were refrozen after thawing to prevent self-aggregation.

Congo Red Preparation

Congo Red (AMRESCO, Solon OH) was prepared at a stock concentration of 10 mM in OR-2 with Ca^{2+} (pH 7.5 with NaOH) and stored at room temperature in order to prevent formation of micelles. CR was filtered a 0.2 um cellulose acetate sterile syringe filter immediately prior to use.

Acetylcholine

Acetylcholine solutions were prepared fresh each week from powdered ACh (Sigma, St. Louis MO) in OR-2 with Ca^{2+} (pH 7.5) at a stock concentration of 10 mM. Stock concentration was diluted to the desired concentration prior to application.

RNA Preparation

Human α7 (Origene # SC124074-20) subunit genes in the pCMV6-XL4 plasmid (Origene Technologies, Rockville MD) were transformed in One Shot® E. coli chemically competent cells (Invitrogen, Carlsbad CA) according to the protocol supplied by the manufacturer. Plasma isolation and purification were performed using the HiSpeed® plasmid purification kit by QIAGEN Inc. (Valencia CA). Plasmids containing the α7 genes were linearized with Xba1 according to the protocol recommended by manufacturer. The mRNA was then transcribed, capped on the 5' end and PolyA tail was added. LiCl purification was performed using the mMessage mMachine® T7 Ultra Kit (Ambion, Carlsbad CA) according to the protocol provided by the manufacturer. The α7 mRNA was resuspended in TE Buffer (Bioexpress, Layton UT), aliquoted and stored at -80 degrees Celsius until used.

Frog Care and Oocyte Preparation

Female *Xenopus laevis* frogs (Xenopus 1, Inc. Dexter MI) were housed under 12-h day/night cycle at temperatures maintained from 17-19 degrees Celsius. Surgeries were performed once a week under sterile conditions and in accordance with the IACUC protocol. Prior to surgery frogs were anesthetized by immersion in a solution (12mM NaCl, 0.134 mM KCl, 0.181 $CaCl_2$, 0.476 mM $NaHCO_3$, 5mM HEPES, adjusted to pH 7.5 with NaOH) containing

0.1% ethylmetaaminobenzoate (MS-222; Sigma, St. Louis MO) for 15 minutes until unresponsive. Ovary sacs were removed and placed in a Ca^{2+}-free OR-2 solution (89.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ anhydrous, 5 mM HEPES, adjusted to pH 7.5 with NaOH), and manually separated with forceps. The follicular layer was removed using two different methods. Oocytes were either defolliculated by treatment with collagenase A (Sigma, St. Louis MO) for 2 hours on a tilting table at 17-19 degrees Celsius or manually defolliculated with fine-tip forceps under a microscope at 12x magnification. The cells were then rinsed with the Ca^{2+}-free OR-2 solution and subsequently stored in either an OR-2 solution with Ca^{2+} and theophylline or the same solution supplemented with Pen Strep (Invitrogen) at 14-17 degrees Celsius for a minimum of 24 hours prior to injection.

Additional oocytes were purchased for injection and recording from EcoCyte Bioscience (Austin TX). Oocytes were shipped in Barth's solution (88 mM NaCl, 1 mM KCl, .33 mM $Ca(N0_3)_2$ x $4H_2O$, .41 mM $CaCl_2$ x $2H_2O$, .82 mM $MgSO_4$ x $7H_2O$, 5 mM Tris-HCl, 2.4 mM $NaHCO_3$) supplemented with Pen Strep (100 µg/mL).

RNA Expression in *Xenopus* Oocytes

A nanojet II automatic nanoliter injector (Drummond Scientific, Broomall PA) was used to inject human α7 mRNA into oocytes. Each oocyte was injected with 50.6 nL of mRNA for a total concentration of 75.9 ng of α7 mRNA per oocyte. Following injection the oocytes were either stored in an OR-2 Ca^{2+} with theophylline solution with or without Pen Strep at 14-17 degrees Celsius. Solution was changed daily, and recordings were obtained 3-5 days following injections.

Electrophysiology

Recordings of oocyte response to ACh and Aβ were measured via two-electrode voltage clamp (TEVC) electrophysiology using a standard Geneclamp 500B amplifier (Axon Instruments, Sunnyvale CA) and a Digidata 1322A digitizer (Axon Instruments, Sunnyvale CA). Traces were recorded using Clampex 9.2 software (Axon Instruments, Sunnyvale CA) and analyzed on ClampFIit 9.2 (Axon Instruments, Sunnyvale CA).

Electrodes containing 3M KCl and having a resistance between 1 and 10 MΩ were made from 1.5 mm borosilicate glass capillaries (Harvard Apparatus, Kent, England) pulled on a Flaming/Brown micropipette puller model P-97 (Sutter Instrument, Novato, CA). Holding potentials were clamed at -60mV unless otherwise stated, and traces were sampled at 5 KHz and filtered at 1000 Hz.

Oocytes were stabilized on a perfusion platform (model RC-1Z Warner Instruments, Hamden CT) and continuously perfused with OR-2 with Ca^{2+} at 15-18 mL/minute. Administration of OR-2 with Ca^{2+}, ACh and Aβ was controlled by and automated perfusion system (Valvelink 8 Automate Scientific Inc., Berkley CA). All electrophysiological experiments were performed at room temperature. Aβ was also applied using the same perfusion system with subsequent exposure to either the 333µM or 1000µM concentrations of ACh. The length of exposure to ACh and Aβ varied as the experiment dictated.

Unless otherwise stated, recording protocol consisted of five 2-minute sweeps. The sequence of solution administrations within those sweeps was as follows (cell clamped at -60 mV):

1. 5 sec OR-2 with Ca^{2+}

2. 7 sec OR-2 with Ca^{2+} from control valve
3. 10 sec OR-2 with Ca^{2+}
4. 7 sec ACh (333 µM unless otherwise indicated)
5. 91 sec OR-2 with Ca^{2+} washout

The above sequence of events was preceded by 30 sec, 1 min, 2 min, 3 min, 4 min or 5 min perfusion with Aβ where indicated.

Congo Red Spectroscopic Assay

Stock solution of 10 mM CR was filtered and diluted in OR-2 with Ca^{2+} to a concentration of 50 µM. UV-Vis spectrophotometer was zeroed between 400 and 700 nm at room temperature with a 1.5 µL sample of OR-2 with Ca^{2+}. A 5 µL sample of OR-2 with Ca^{2+} was added to 5 µL of the 50 µM CR solution and briefly triturated by pipetting the solution up and down. A 1.5 µL sample of the CR/OR-2 solution was scanned between 400 and 700 nM and the spectrum recorded. The same process was repeated with 5 µL of Aβ42 sample added to 5 µL of 50 µM CR and allowed to incubate at room temperature for 30 min after which the spectrum was recorded. In order to insure that no residual CR/Aβ solution was transferred between measurements, the absorbance of the OR-2 solution used as a blank was run between each sample. The Congo Red spectrum was analyzed in order to detect the presence or absence of a maximal spectral difference at 540 nM.

Absorbance Assay (280 nm)

An absorbance assay was run using A280 program (version 3.5.1) on a NanoDrop ND-1000 spectrophotometer (Wilmington DE). The spectrophotometer was zeroed with 2 µL OR-2

buffer solution, and the absorbance of 2 µL of the peptide solution was measured at 280 nm. Aβ42 peptide concentrations were calculated using the molar extinction coefficient (Ɛ) for tyrosine (tyr; 1280 $M^{-1}cm^{-1}$).

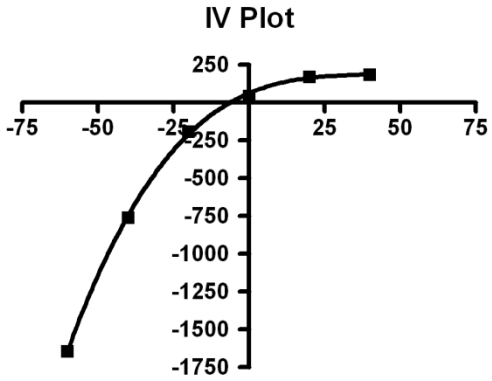
Data Analysis

InStat ver. 3.05, GraphPad software (San Diego CA) was used to perform repeated measures ANOVA statistical analysis. Statistical significance was determined by $p< 0.05$.

RESULTS

IV Plot

In order to verify expression and characterize the nAChR expressed in the Xenopus oocytes, an I/V plot was obtain by clamping the membrane potential at -60 mV, -40 mV, -20 mV, 0, 20 mV and 40 mV and measuring the respective currents upon application of 333 µM ACh. The I/V plot was found to be consistent with that of an α7 nAChR as described in the literature (Fig. 5a).



Acetylcholine Dose Response Curve

Naïve oocytes, not injected with α7 mRNA, were exposed to ACh according to the protocol previously outlined. Naïve oocytes not expressing the receptor exhibited no ACh-induced depolarization (figure not shown).

We did, however, see the characteristic ACh-induced response in oocytes injected with α7 mRNA with the downward current indicative of the influx of Ca^{2+} ions into the cell via the open cation pore. To further characterize the receptor subtype, a dose-response curve was obtained by exposing the cell to 10 uM, 33 uM, 100 uM, 333 uM and 100 uM ACh as previously described. Peak amplitudes were measured at each concentration and normalized to the average peak response to 1000 uM ACh. An EC_{50} of 57 µM

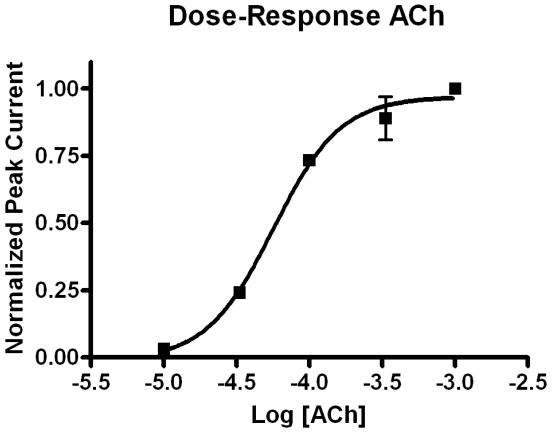


Figure 5a: The I/V plot for a7 nAChRs expressed in Xenopus oocytes. The reversal potential for the channels was calculated to be -6 mV.

Figure 5b: The dose response curve to ACh of α7 nAChRs expressed in *Xenopus laevis* oocytes. The EC_{50} is 57 uM (r^2=.9721, Hill coefficient=1.889).

(r^2=.9721, Hill coefficient=1.889) was observed based on the dose-response curve (Fig. 5b). This represents normal values for the α7 nAChR subtype and further confirms the expression of the receptor (Briggs and McKenna 1996).

Aβ Inhibition of nAChRs as a Function of Exposure Time

Previously in our lab, it was determined that Aβ-induced inhibition of nAChRs was most significant and consistently observed with extended applications (5 minutes) of 30 nM Aβ.

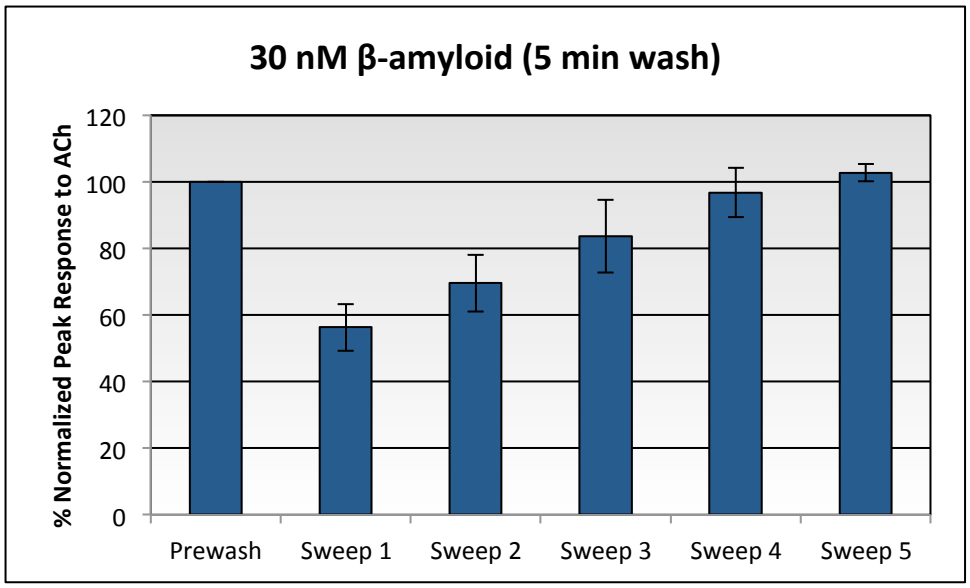


Figure 6: Aβ-induced inhibition of nAChRs by 30 nM Aβ and subsequent recovery of full ACh response following washout.

Maximum inhibition was seen with the first sweep, followed by gradual recovery of the peak amplitude by sweep 5, indicating that the Aβ-induced inhibition is reversible (Fig.6). Based on these findings, a concentration of 30 nM Aβ was used in all amyloid/nAChR experiments.

While we have determined the dose-dependency of the Aβ inhibition in previous experiments, the timing of the block has yet to be closely examined in our lab. Because the Aβ inhibition of the receptor possibly occurs by mechanisms besides simple competion at a binding

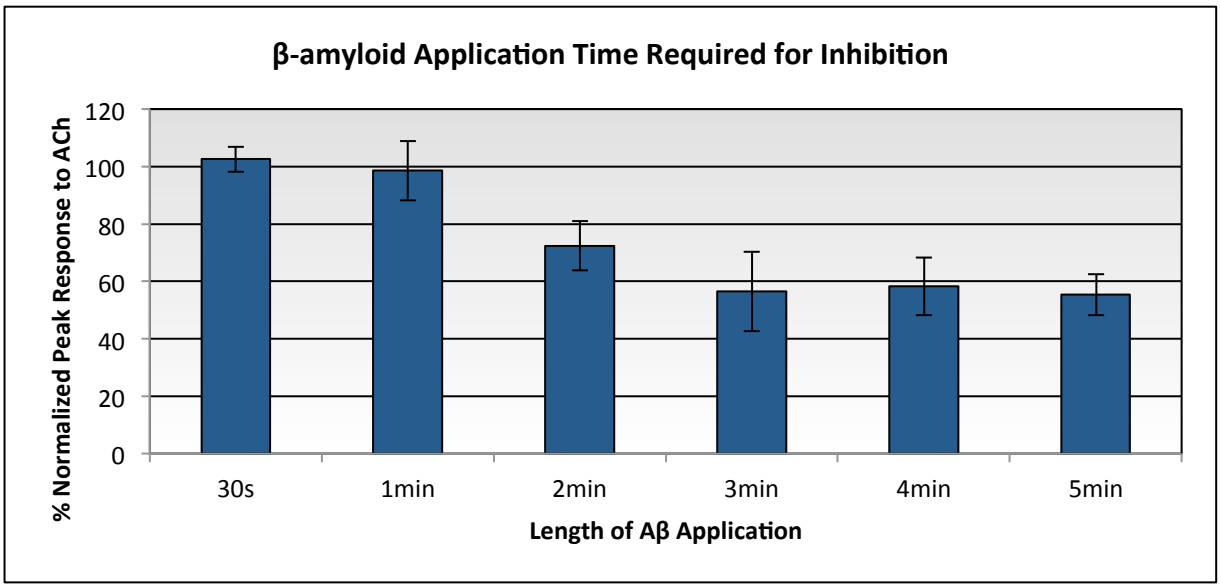


Figure 7: Time-dependent inhibition of nAChRs by Aβ. Two minute application was required for onset of Aβ-induced inhibition

site, we decided to examine the time required to induce the Aβ block. Aβ perfusion was administered for 30 sec, 1 min, 2 min, 3 min, 4 min and 5 min, and peak amplitudes were compared as a function of length of Aβ exposure (Fig.7). Inhibition was not seen in the oocytes exposed for just 30 sec or even 1 min. The inhibitory effect was not observed until at least a 2 min Aβ wash was applied, at which point, the peak response amplitude was reduced by 30%. A maximum inhibition of almost 50% was observed as a result of the 3 min Aβ wash. This data is consistent with the idea that the Aβ-induced inhibition on nAChRs is a function of the initiation of signaling events within the cell and agrees with the findings that activation of the JNK/MAPK and ERK/MAPK pathways is time-dependent (Bell, O'Riordan et al. 2004; Buckingham, Jones et al. 2009).

Possible Reduction of Aβ Inhibition in Presence of Antibiotics

Due to the unpredictability of Aβ, reproducing data has proven to be challenging at times. To enhance the health of our oocytes, we began to supplement the OR-2 with Ca^{2+}

solution with antibiotics. While the addition of antibiotics to our holding solutions seemed to improve oocyte health, it also seemed to coincide with a sudden inability to elicit the same amyloid-induced inhibition we had seen consistently in our experiments (Fig.8b).

30 nM β-amyloid for 2 min

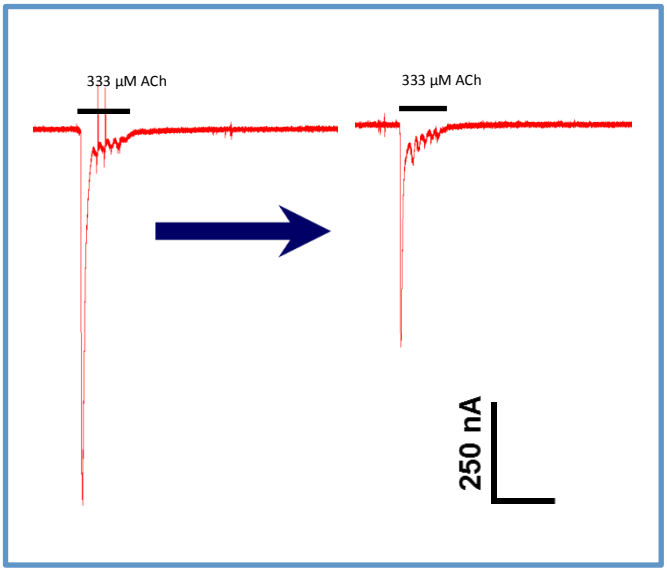


Figure 8a: Typical Aβ-induced inhibition of ACh current.

Incubation with Pen Strep + 30 nM β-amyloid for 2 min

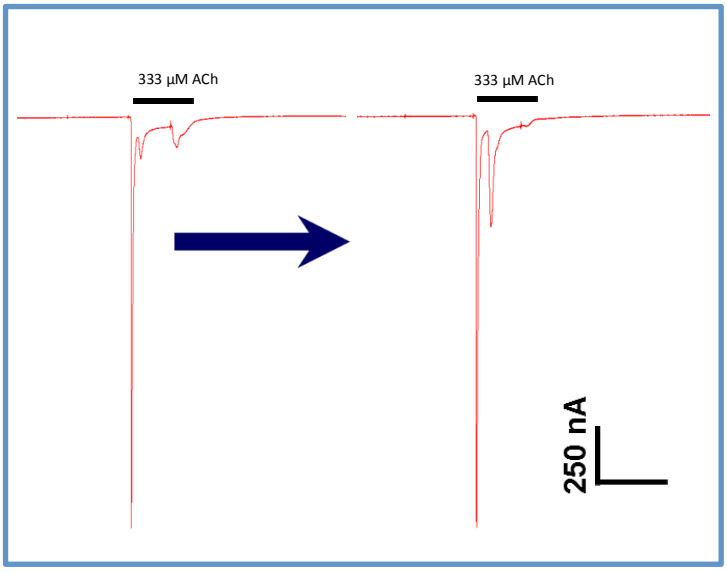


Figure 8b: Incubation of the oocytes in Pen Strep solution prevents 30 nM Aβ blockade of receptors.

To test whether or not the antibiotic interfered with the Aβ/ACh interaction, we began to store a portion of our oocytes in antibiotic-free solution to see if the amyloid inhibition of nAChRs might be restored. Indeed, upon removal of the antibiotic from the OR-2 storage solution, the Aβ inhibition was once again observed as expected (Fig.8a). Unfortunately, this was never confirmed on oocytes from the same surgery but was observed in oocytes from different batches exposed to the same Aβ preparation. Attempts to reproduce this data in oocytes from the same surgery continually failed due to different variables that are being addressed by standardizing Aβ preparation and handling.

A280 Absorbance Assay

To address the variability between our Aβ preparations, we performed a series of assays designed to characterize and quantify the composition of peptides in our Aβ solution. First, we employed the A280 absorbance assay to verify that the lyophilized peptide had indeed been solubilized and was present in our solution preparations. Detecting peptide concentrations using the A280 absorption technique requires the presence of tyrosine (Tyr, Y) and/or tryptophan (Trp, W) residues, which, due to their aromaticity, absorb UV light at 280 nm. Because Aβ contains a tyrosine residue, and the molar extinction constant of Tyr (1280 $M^{-1}cm^{-1}$) is known, Beer's law can be applied to calculate peptide molarity using the following calculation where c represents the concentration of the sample in mg/mL, Ɛ represents the molar extinction constant and A_{280} the UV absorption of the sample at 280 nm:

$$c=A_{280}/\varepsilon$$

In order to verify the presence and quantity of Aβ peptide in solution, this A280 technique was applied to two different fresh preparations of the amyloid peptide reconstituted in two different solvents (dH_2O and OR-2 with Ca^{2+}) and diluted to varying concentrations (10 µM, 1 µM and 500 nM).

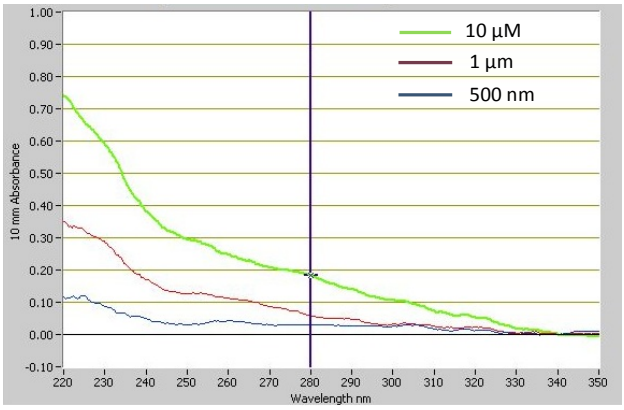


Figure 9: UV absorbances at 280 nm for 10 µM, 1µM and 500 nM preparations of Aβ solution.

Reconstitution of desiccated peptide varies from lab to lab, with some labs dissolving it directly in buffer solution, others in distilled water or basic solutions such as ammonium hydroxide. Up to this point we have favored the reconstitution in buffer solution with mixed results. To test

the ability of the peptide to dissolve in water relative to buffer solutions, we measured the concentration of peptides in solution and compared that to our theoretical concentrations. Additionally, some labs recommend storing the Aβ at stock concentrations in the µM range, while others recommend concentrations less than 1 µM due to the tendency of the peptide to self-aggregate at higher concentrations. To test which theoretical concentration favors actual Aβ concentration similar to what we expect percent yield for several different concentrations of Aβ peptide solution was calculated and compared. Additionally, to test the impact of temperature on the Aβ composition, the preparations were stored either at room temperature or -80 degrees Celsius.

Previous preparations of Aβ stored at stock concentrations of 500 nM in a -80 degree Celsius freezer showed no absorption at 280 nM (data not shown). The fresh preparation of Aβ did, however, exhibit absorption at 280 nm, albeit very minimal (fig.9). Calculated concentrations revealed peptide molarities far below what was expected from the reconstitution of amyloid peptide, with the 10 µM Aβ solution producing the lowest percent yield (Fig.10).

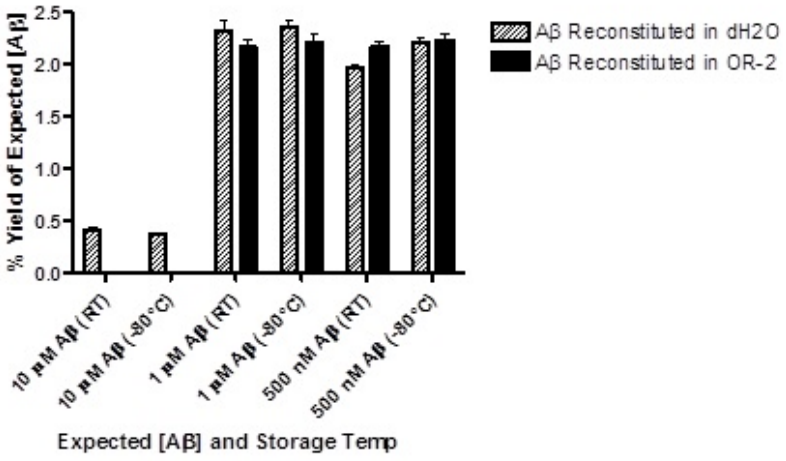


Figure 10: Percent yield of expected Aβ concentration as a function of solvent, temperature and Aβ concentration.

There are several possible explanations for the unexpectedly low yield which will be addressed in the discussion section. One possible explanation that merits discussion is the aggregation of the amyloid into fibrils, which can skew the A280

absorbance reading, as the assay is designed to measure just the soluble forms of the peptide. The more fibrils formed, the lower the absorbance one might expect to see. This might explain why the percent yield in the higher 10 µ Aβ solution yielded such low actual soluble amyloid concentration of the lyophilized peptide. Additionally, variations in salt content of the solvent, in this case OR-2 with Ca^{2+}, can affect the ability of the lyophilized peptide to fully dissolve in the solvent due to ionic interactions.

Congo Red Assay

One variable that has been shown to affect both absorbance at 280 nm as well as the interaction between Aβ and nAChRs is the level of Aβ aggregation in solution. To test the propensity for aggregation in our preparations and to see if aggregation might be the reason behind the lower than expected A280, we tested our samples for spectral shifts that indicated amyloid fibrillation. We attempted to induce aggregation in by incubating them at room temperature for two days, a method that had been shown to increase fibrillation. However, upon repeated analysis using the Congo Red assay, none of the samples significantly exhibited the spectral shift at 540 nm indicative of the presence of Aβ aggregation. A false negative is a possibility when examining sample at such low concentrations.

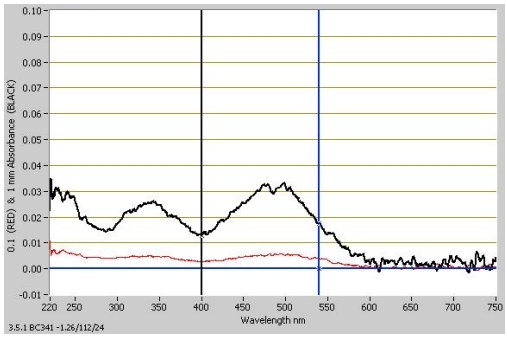


Figure 11: CR+OR-2 control, exhibiting peak absorbance between 490 and 500 nm.

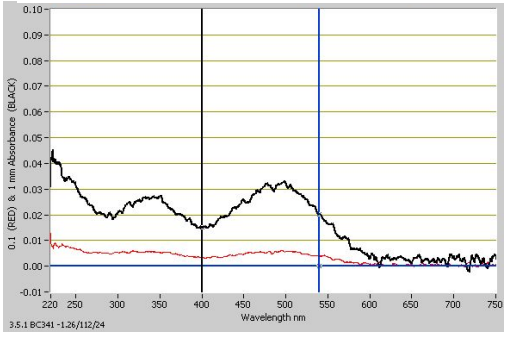


Figure 12: CR+100uM Aβ exhibited a similar absorbance peak between 490 and 500 nm, indicating the absence of a spectral shift and amyloid fibril formation.

DISCUSSION

While important advancements continue to be made in Alzheimer's research, consolidating and making sense of all of the available data regarding the proposed interaction between nAChRs, Aβ is a monumental task. Many factors affect the dynamics of this system, and much of the discrepancies contained within the literature point to lack of standardization of amyloid handling. Much of this is due to the simple lack of understanding of the Aβ molecule itself, but additional measures could be taken to standardize procedure as much as possible.

The instability of Aβ in solution is affected by numerous factors that must be controlled in an experimental setting. One of those factors, that I feel we should address in our lab is the impact of solvent composition on Aβ solubility and aggregation. The inconsistencies in or results and the results of other labs could simply be attributed to lack of understanding of the solvent/solute relationship. Comprehending that relationship is of particular importance in establishing consistent and repeatable results (Snyder, Ladror et al. 1994; Nilsson 2004). Any possible variable that destabilizes the desired oligomeric isoform of the molecule should be examined and addressed.

Aside from the effect that our solvent might have on Aβ solubility, other explanations exist that might shed light on the obstacles faced in our experiments. Despite the results indicated by the Congo Red assay, much of the difficulty we are experiencing points to the aggregation of fibrils in our Aβ solution. The lower than anticipated concentrations of Aβ seen in both the distilled water and OR-2 is concerning and may be due to several different factors. Aside from experimenter error, peptide impurities and solvent composition, another factor not already mentioned is the ability of the NanoDrop spectrophotometer to measure peptides or

fibrils at such low concentrations. In fact, a higher than recommended ratio of CR to peptide solution had to be used in the Congo Red Assay due to the inability of the equipment to clearly record absorbance at CR concentrations at or below 5 µM. Even though the Congo Red assay indicated a lack of fibrillation, various evidence points to it as a possible explanation for the lack of reproducibility in our experimentation. The lower than recommended concentration of Aβ used in the Congo Red Assay should definitely be considered, and the possibility of fibril formation within our preparations should not be eliminated based on our results. With that said, considering the low concentrations that we use in our Aβ protocol, the presence of Aβ fibrils in our solutions might be better detected by alternative methods such as circular dichroism or fluorescence assays.

With that said, despite the difficulty we've experienced in repeating results, the questions that arise from those results are valid and compelling and merit additional examination. The effect of antibiotics on the Aβ/nAChR dynamic could have far-reaching implications, including pharmacological applications. If the unpredictable nature of the Aβ could be controlled, there is a number of interesting research avenues that could be pursued with regards to antibiotics.

However, possibly the most promising avenue in researching the role of Aβ in cytotoxicity is that of signaling cascades. Of particular interest is the tyrosine kinase, FYN, whose exact role in neuroprotection or neurodegeneration is not well understood but seems to be an important factor in the Aβ/nAChR relationship. Examining that role within the parameters of our capabilities and resources and experimental design is a compelling possibility.

The literature indicates the significance of the phosphorylation state of the receptor in its ability to function optimally. The possibility that FYN and other SFKs are responsible for the phosphorylation state of α7 nAChRs, and that changes in FYN and SFK activity are seen in the presence of α7 nAChR agonists as well as in the presence of Aβ, implicates FYN as a possible downstream molecule involved in the pathogenicity of Aβ. The potential role of a second messenger cascade is substantiated by our experimental data which indicates a delay in the response of α7 nAChRs to Aβ. The idea that the Aβ effect is not immediate is consistent with the hypothesis that the inhibition of nAChRs is more than just a simple, direct physical blockade of the ion pore, but that the inhibition might be attributable to alterations in second messenger signaling cascades feeding back onto the nAChR. It seems feasible that Aβ could initiate phosphorylation of the α7 nAChR by FYN or another SFK, thus reducing its responsiveness to ACh. A reduction in responsiveness to its agonists could very well represent the initial stages of the cholinergic dysfunction characteristic of AD. The deleterious effects of altering signaling cascades might also explain the various symptoms exhibited by Alzheimer's sufferers, including memory loss, long-term potentiation (LTP) inhibition, irritability, etc. A focus of our continuing research should be the changes seen in the signaling cascade, specifically the phosphorylation state of the α7 receptor, due to nAChR/Aβ interaction. Moving forward we could easily apply some of the methods employed by Charpantier to our experimental protocol as we seek to examine the specific mechanisms behind the Aβ-induced inhibition of α7 nAChRs. But once again, our ability to further our research depends upon our ability to control the Aβ peptide.

REFERENCES

Albuquerque, E. X., E. F. Pereira, et al. (1997). "Nicotinic acetylcholine receptors on hippocampal neurons: distribution on the neuronal surface and modulation of receptor activity." J Recept Signal Transduct Res **17**(1-3): 243-266.

Albuquerque, E. X., E. F. Pereira, et al. (1998). "Neuronal nicotinic receptors modulate synaptic function in the hippocampus and are sensitive to blockade by the convulsant strychnine and by the anti-Parkinson drug amantadine." Toxicol Lett **102-103**: 211-218.

Amici, M., F. Eusebi, et al. (2005). "Effects of the antibiotic gentamicin on nicotinic acetylcholine receptors." Neuropharmacology **49**(5): 627-637.

Arias, E., E. Alés, et al. (2004). "Galantamine prevents apoptosis induced by β-amyloid and thapsigargin: involvement of nicotinic acetylcholine receptors." Neuropharmacology **46**(1): 103-114.

Barrantes, F. J., V. Borroni, et al. (2010). "Neuronal nicotinic acetylcholine receptor-cholesterol crosstalk in Alzheimer's disease." FEBS Lett **584**(9): 1856-1863.

Bell, K. A., K. J. O'Riordan, et al. (2004). "MAPK recruitment by β-amyloid in organotypic hippocampal slice cultures depends on physical state and exposure time." Journal of Neurochemistry **91**(2): 349-361.

Briggs, C. A. and D. G. McKenna (1996). "Effect of MK-801 at the human α7 nicotinic acetylcholine receptor." Neuropharmacology **35**(4): 407-414.

Buckingham, S. D., A. K. Jones, et al. (2009). "Nicotinic Acetylcholine Receptor Signalling: Roles in Alzheimer's Disease and Amyloid Neuroprotection." Pharmacological Reviews **61**(1): 39-61.

Buckingham, S. D., A. K. Jones, et al. (2009). "Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection." Pharmacol Rev **61**(1): 39-61.

Charpantier, E., A. Wiesner, et al. (2005). "α7 Neuronal Nicotinic Acetylcholine Receptors Are Negatively Regulated by Tyrosine Phosphorylation and Src-Family Kinases." The Journal of Neuroscience **25**(43): 9836-9849.

Cho, C.-H., W. Song, et al. (2005). "Rapid Upregulation of α7 Nicotinic Acetylcholine Receptors by Tyrosine Dephosphorylation." The Journal of Neuroscience **25**(14): 3712-3723.

Conejero-Goldberg, C., P. Davies, et al. (2008). "Alpha7 nicotinic acetylcholine receptor: A link between inflammation and neurodegeneration." Neuroscience and Biobehavioral Reviews **32**(4): 693-706.

Dajas-Bailador, F. and S. Wonnacott (2004). "Nicotinic acetylcholine receptors and the regulation of neuronal signalling." Trends in Pharmacological Sciences **25**(6): 317-324.

Dougherty, J. J., J. Wu, et al. (2003). "Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex." J Neurosci **23**(17): 6740-6747.

Ellis, J. R., V. L. Villemagne, et al. (2008). "Relationship between nicotinic receptors and cognitive function in early Alzheimer's disease: a 2-[18F]fluoro-A-85380 PET study." Neurobiol Learn Mem **90**(2): 404-412.

Galzi, J. L., S. Bertrand, et al. (1996). "Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor." EMBO J **15**(21): 5824-5832.

Glabe, C. G. (2008). "Structural Classification of Toxic Amyloid Oligomers." Journal of Biological Chemistry **283**(44): 29639-29643.

Gotti, C. and F. Clementi (2004). "Neuronal nicotinic receptors: from structure to pathology." Progress in Neurobiology **74**(6): 363-396.

Gotti, C., M. Moretti, et al. (2006). "Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation." Neurobiology of Disease **23**(2): 481-489.

Gotti, C., M. Zoli, et al. (2006). "Brain nicotinic acetylcholine receptors: native subtypes and their relevance." Trends in Pharmacological Sciences **27**(9): 482-491.
Hardy, J. and D. J. Selkoe (2002). "The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics." Science **297**(5580): 353-356.
Hellstrom-Lindahl, E., M. Mousavi, et al. (1999). "Regional distribution of nicotinic receptor subunit mRNAs in human brain: comparison between Alzheimer and normal brain." Brain Res Mol Brain Res **66**(1-2): 94-103.
Hernandez, C. M., R. Kayed, et al. (2010). "Loss of alpha7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease." J Neurosci **30**(7): 2442-2453.
Hogg, R. C., M. Raggenbass, et al. (2003). "Nicotinic acetylcholine receptors: from structure to brain function." Reviews of Physiology, Biochemistry and Pharmacology, Vol 147 2003 **147**: 1-46.
Ikonomovic, M. D., L. Wecker, et al. (2009). "Cortical alpha 7 Nicotinic Acetylcholine Receptor and beta-Amyloid Levels in Early Alzheimer Disease." Archives of Neurology **66**(5): 646-651.
Kihara, T., S. Shimohama, et al. (2001). "α7 Nicotinic Receptor Transduces Signals to Phosphatidylinositol 3-Kinase to Block A β-Amyloid-induced Neurotoxicity." Journal of Biological Chemistry **276**(17): 13541-13546.
Klunk, W. E., R. F. Jacob, et al. (1999). "Quantifying Amyloid β-Peptide (Aβ) Aggregation Using the Congo Red-Aβ (CR–Aβ) Spectrophotometric Assay." Analytical Biochemistry **266**(1): 66-76.
Kumar-Singh, S., J. Theuns, et al. (2006). "Mean age-of-onset of familial alzheimer disease caused by presenilin mutations correlates with both increased Abeta42 and decreased Abeta40." Hum Mutat **27**(7): 686-695.
Li, S.-F., M.-N. Wu, et al. (2011). "Requirement of α7 nicotinic acetylcholine receptors for amyloid beta protein-induced depression of hippocampal long-term potentiation in CA1 region of rats in vivo." Synapse **65**(11): 1136-1143.
Liu, R. Y., R. Gu, et al. (2007). "[Influence of beta-amyloid protein and cholesterol on the pathological changes of Alzheimer's disease and expression of nicotinic acetylcholine receptors in rats]." Zhonghua Bing Li Xue Za Zhi **36**(3): 184-189.
Luheshi, L. M., G. G. Tartaglia, et al. (2007). "Systematic in vivo analysis of the intrinsic determinants of amyloid Beta pathogenicity." PLoS Biol **5**(11): e290.
Nilsson, M. R. (2004). "Techniques to study amyloid fibril formation in vitro." Methods **34**(1): 151-160.
Nordberg, A. (2001). "Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications." Biol Psychiatry **49**(3): 200-210.
Parri, H. R., C. M. Hernandez, et al. (2011). "Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease." Biochem Pharmacol **82**(8): 931-942.
Parri, R. H. and T. K. Dineley (2010). "Nicotinic acetylcholine receptor interaction with beta-amyloid: molecular, cellular, and physiological consequences." Curr Alzheimer Res **7**(1): 27-39.
Pettit, D. L., Z. Shao, et al. (2001). "beta-Amyloid(1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice." J Neurosci **21**(1): RC120.
Rothlin, C. V., E. Katz, et al. (2000). "Block of the α9 nicotinic receptor by ototoxic aminoglycosides." Neuropharmacology **39**(13): 2525-2532.
Schmit, J. D., K. Ghosh, et al. (2011). "What Drives Amyloid Molecules To Assemble into Oligomers and Fibrils?" Biophysical Journal **100**(2): 450-458.
Snyder, S. W., U. S. Ladror, et al. (1994). "Amyloid-beta aggregation: selective inhibition of aggregation in mixtures of amyloid with different chain lengths." Biophys J **67**(3): 1216-1228.
Sudweeks, S. N. and J. L. Yakel (2000). "Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons." J Physiol **527 Pt 3**: 515-528.

Tang, X., Y. Feng, et al. (2007). "Src-family tyrosine kinase fyn phosphorylates phosphatidylinositol 3-kinase enhancer-activating Akt, preventing its apoptotic cleavage and promoting cell survival." Cell Death and Differentiation **14**(2): 368-377.

Tang, Z., Y. An, et al. (2008). "Inhibiting gene expression of alpha3 nicotinic receptor in SH-SY5Y cells with the effects on APP metabolism and antioxidation in Alzheimer's disease." Neurochem Int **53**(5): 112-117.

Teplow, D. B. (2006). Preparation of Amyloid β-Protein for Structural and Functional Studies. Methods in Enzymology. K. Indu and W. Ronald, Academic Press. **Volume 413:** 20-33.

Walsh, D. M. and D. J. Selkoe (2007). "A beta oligomers - a decade of discovery." J Neurochem **101**(5): 1172-1184.

Wang, H.-Y., D. H. S. Lee, et al. (2000). "β-Amyloid1–42 Binds to α7 Nicotinic Acetylcholine Receptor with High Affinity: IMPLICATIONS FOR ALZHEIMER'S DISEASE PATHOLOGY." Journal of Biological Chemistry **275**(8): 5626-5632.

Wang, H.-Y., W. Li, et al. (2003). "{alpha}7 Nicotinic Acetylcholine Receptors Mediate {beta}-Amyloid Peptide-induced Tau Protein Phosphorylation." J. Biol. Chem. **278**(34): 31547-31553.

Wang, H.-Y., A. Stucky, et al. (2009). "Dissociating β-Amyloid from α7 Nicotinic Acetylcholine Receptor by a Novel Therapeutic Agent, S 24795, Normalizes α7 Nicotinic Acetylcholine and NMDA Receptor Function in Alzheimer's Disease Brain." The Journal of Neuroscience **29**(35): 10961-10973.

Wevers, A., L. Burghaus, et al. (2000). "Expression of nicotinic acetylcholine receptors in Alzheimer's disease: postmortem investigations and experimental approaches." Behav Brain Res **113**(1-2): 207-215.

Wu, C., J. Scott, et al. (2012). "Binding of Congo Red to Amyloid Protofibrils of the Alzheimer A²9 40 Peptide Probed by Molecular Dynamics Simulations." Biophysical journal **103**(3): 550-557.

Zolles, G., E. Wagner, et al. (2009). "Functional expression of nicotinic acetylcholine receptors in rat neocortical layer 5 pyramidal cells." Cereb Cortex **19**(5): 1079-1091.

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Set up experiments and exercises in advanced physiology including computer simulations of muscle function, endocrine disease, and human physiology
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Research Interests

Aug 2010-present

I am currently involved in researching the interactions between B-amyloid proteins and various nicotinic receptor subtypes typically found in the hippocampus of the brain. I am responsible for preparing mRNA and injecting it into frog oocytes. Following expression, I characterize the receptors expressed via two electrode voltage clamping (TEVC).

Aug 2004-Apr 2005

I was able to participate in research revolving around the effect of a high sodium diet on the periventricular nucleus (PVN) of rats. I was primarily responsible for creating rat brain slices using the Cryostat and slide preparation.

Volunteer Experience

- 2 year LDS mission in Carlsbad, CA (Spanish speaking)
- Volunteer tutor at BYU
  - Currently tutor fellow students in chemistry, biology, physiology
- Utah Special Olympics volunteer
  - Served as committee head for fundraising and opening ceremonies for Utah's summer games
- Illinois Special Olympics volunteer
  - Helped organize dental exams for the participants
- Volunteer Spanish translator for Give Kids a Smile at SIUE SDM
- Volunteer judge for the 2012 Central Utah Science and Engineering Fair (CUSEF)

Extracurricular Activities

- Officer in BYU's Neuroscience Club
- Intramural sports (basketball, football, softball, water polo and volleyball)
- Avid reader
- Avid runner
    - Completed a 180-mile, 24-hour relay race with a twelve member team

Miscellaneous

- Officer in BYU's Neuroscience Club
- Fluent in Spanish
- CRLA level 1 certified tutor