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Journal

Biochemistry, 54(50)

ISSN

0006-2960

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Publication Date

2015-12-22

DOI

10.1021/acs.biochem.5b00988

Peer reviewed

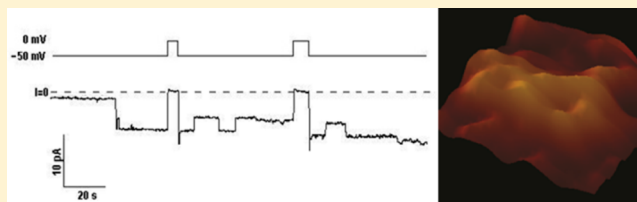
Ion Channel Formation by Tau Protein: Implications for Alzheimer's Disease and Tauopathies

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ABSTRACT: Tau is a microtubule associated protein implicated in the pathogenesis of several neurodegenerative diseases. Because of the channel forming properties of other amyloid peptides, we employed planar lipid bilayers and atomic force microscopy to test tau for its ability to form ion permeable channels. Our results demonstrate that tau can form such channels, but only under acidic conditions. The channels formed are remarkably similar to amyloid peptide channels in their appearance, physical and electrical size, permanence, lack of ion selectivity, and multiple channel conductances. These channels differ from amyloid channels in their voltage dependence and resistance to blockade by zinc ion. These channels could explain tau's pathologic role in disease by lowering membrane potential, dysregulating calcium, depolarizing mitochondria, or depleting energy stores. Tau might also combine with amyloid beta peptides to form toxic channels.



INTRODUCTION

Tau is a microtubule associated protein genetically linked to dementia. It is found in the neurofibrillary tangles of Alzheimer's disease where it appears aggregated as paired helical filaments.^{1–4} Tau is also associated with other neurodegenerative diseases such as fronto-temporal dementia.^{5–8} Tau is intrinsically disordered proteins that is capable of aggregation, especially in the presence of lipid membranes and cause their disruption.^{9–13} The binding is mediated by short amphipathic helices located in the microtubule binding repeat regions of tau.^{10,14–16} Although the role of tau in causing neurodegeneration remains uncertain, it has been demonstrated to show toxicity to neurons in vitro.¹⁷ Tau has also been shown to spread from neuron to neuron in the brain, exhibiting a prion-like contagion.^{18,19}

Amyloid peptides and proteins associated with Alzheimer's disease, prion diseases, Parkinson's disease, and others have been demonstrated to form nonspecific ion channels in lipid membranes.^{20–22} These channels have similar properties that suggest a common mechanism of action in causing cellular pathology. Tau's propensity to aggregate in the presence of membranes and cause membrane disruption led us to examine the effects of tau-441, an isoform of human tau (2N4R, hereafter tau) on planar lipid bilayers.²³ In this report, we present the structure and activity of tau in planar lipid bilayers as examined by electrical conductance and high resolution atomic force microscopy studies.

METHODS

Planar Lipid Bilayer Electrical Recording. Phospholipid bilayer membranes were formed as previously described.²⁴

Briefly, bilayer membranes were formed by placing a bubble of lipid dissolved in solvent onto the end of the Teflon tube approximately 300 μm in diameter. The chamber design allow rapid introduction of solution into immediate proximity with the membrane in a volume of 50 μL . For this, 1% agar salt bridges with 1 M KCl were used to connect the Ag/AgCl electrodes (E-207, Warner Instruments) to the solutions. Voltage clamp conditions were employed in all experiments. The side that tau was added (*cis* side) was taken as ground. All voltages given refer to the voltage of the *trans* side. Current was recorded with an "Axopatch C1" amplifier, and stored by "DataTrax" system for later analysis. Membrane capacitance and resistance were monitored frequently to ensure the integrity of the membranes.

POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine, and POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol), lipids were purchased from Avanti Polar Lipids and stored at $-20\text{ }^\circ\text{C}$. Lipids were dissolved in *n*-heptane to a final concentration of 1.5%. Tau was dissolved in double distilled water at 1 mg/mL concentration and stored frozen in aliquots at $-20\text{ }^\circ\text{C}$. All other chemicals and tau were purchased from Sigma (St. Louis, MO).

AFM Imaging. For AFM experiments, tau was suspended in MES buffer (50 mM 2-(*N*-morpholino) ethanesulfonic acid, 100 mM NaCl, 0.5 mM EGTA, pH 6.8) at 1 mg/mL concentration and stored in 10 μL aliquots at $-20\text{ }^\circ\text{C}$ for one time use in experiments to avoid multiple freeze thaw cycles. 1,

Received: September 7, 2015

Revised: October 22, 2015

Published: November 17, 2015

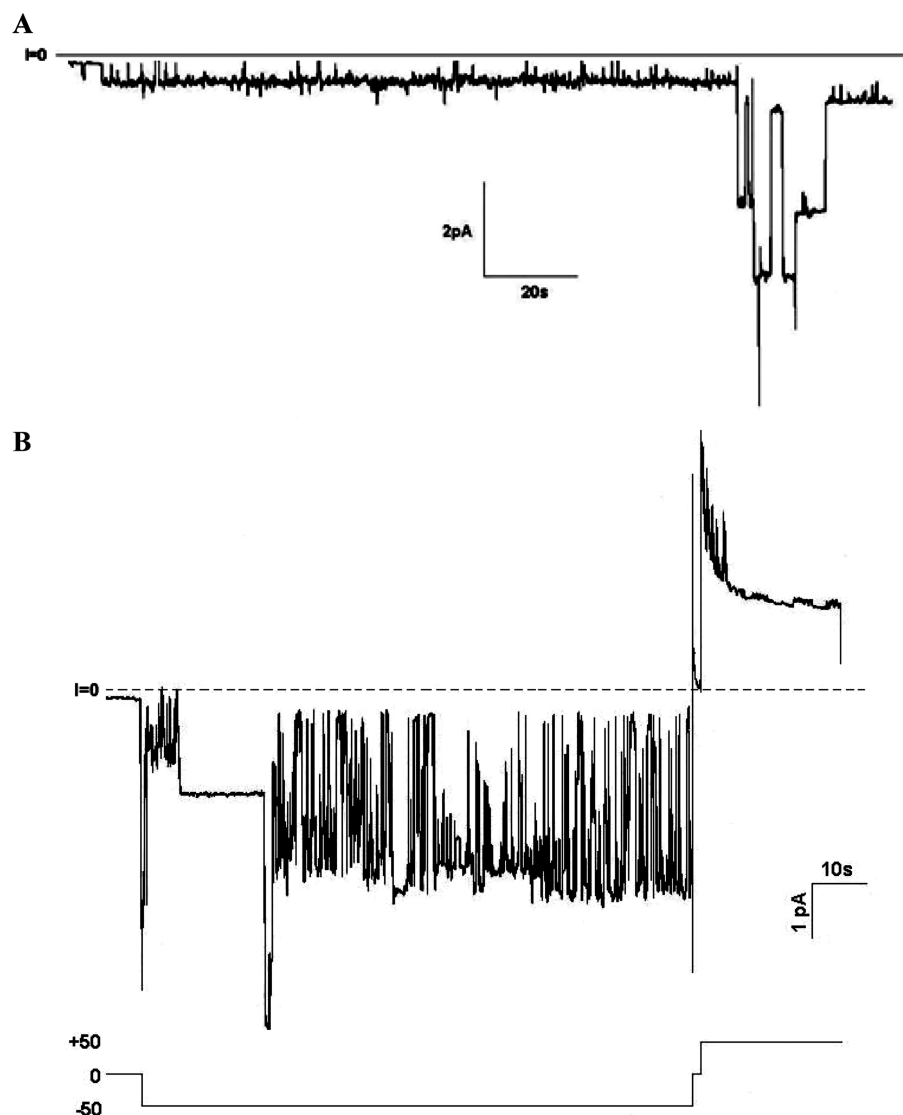


Figure 1. (A) Single channel currents induced by tau in bilayer membrane at -50 mV; 0.43 μM tau in 10 mM Tris-citrate (pH 5.2) was added to the lipid bilayer made up of 2:1 POPE/POPG in 100 mM NaCl. (B) Voltage dependence of currents induced by tau.

2-Dioleoyl-*sn*-glycero-3-phosphoserine (DOPS) phospholipids were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Lipid vesicles were prepared by drying the chloroform stock solution and suspending the phospholipids in a buffer solution (50 mM MES, 100 mM NaCl, 1 mM MgCl_2). The lipid solution was sonicated for 5 min to form vesicles, and tau aliquots were added to lipid vesicles to form proteoliposomes at a 1:100 protein–lipid ratio. The mixture was gently vortexed and sonicated in an ice bath for 15 min.

AFM samples of tau peptide on mica were prepared by depositing 20 μL of protein solution (0.1 mg/mL) onto freshly cleaved mica discs (12 mm diameter). After 2 min, samples were rinsed with 1 mL of Milli-Q water, and dried under a stream of inert gas. AFM samples of tau peptide on planar lipid bilayers were prepared by depositing 50 μL of proteoliposome solution onto freshly cleaved mica. Samples were incubated for 10 or 100 min and gently rinsed thrice with phosphate-buffered saline (PBS). All samples were imaged immediately after preparation. AFM imaging was conducted on a Multimode AFM equipped with a Nanoscope IIIa controller (Bruker, Santa Barbara, CA). Experiments were conducted in air or in liquid

conditions in PBS using cantilevers with nominal spring constants of 0.02 N/m. Image analysis was performed using Bruker Nanoscope Analysis software.

RESULTS

Ion Channel Activity. Figure 1A shows the current flow across the phospholipid bilayer upon addition of tau (32 – 43 μM) to one side of the membrane (*cis* side) when the membrane voltage was held at -50 mV (voltages refer to the *trans* side). In the absence of peptide, baseline current flow was indistinguishable from zero (~ 0.05 pA that gives ~ 1 pS of conductance), indicating that the membrane by itself is impermeable to ions in the solution. Only stable, low conductance, low noise membranes were used for experiments. Channel behavior exhibited voltage dependence, with more channel openings and closings at negative voltage than at positive voltage (Figure 1B). This voltage-dependent behavior was reversible and reproducible.

The induced currents were due to the formation of single channels in the membrane. Figure 2 shows the opening and closing of single channels in the membrane. The single channel

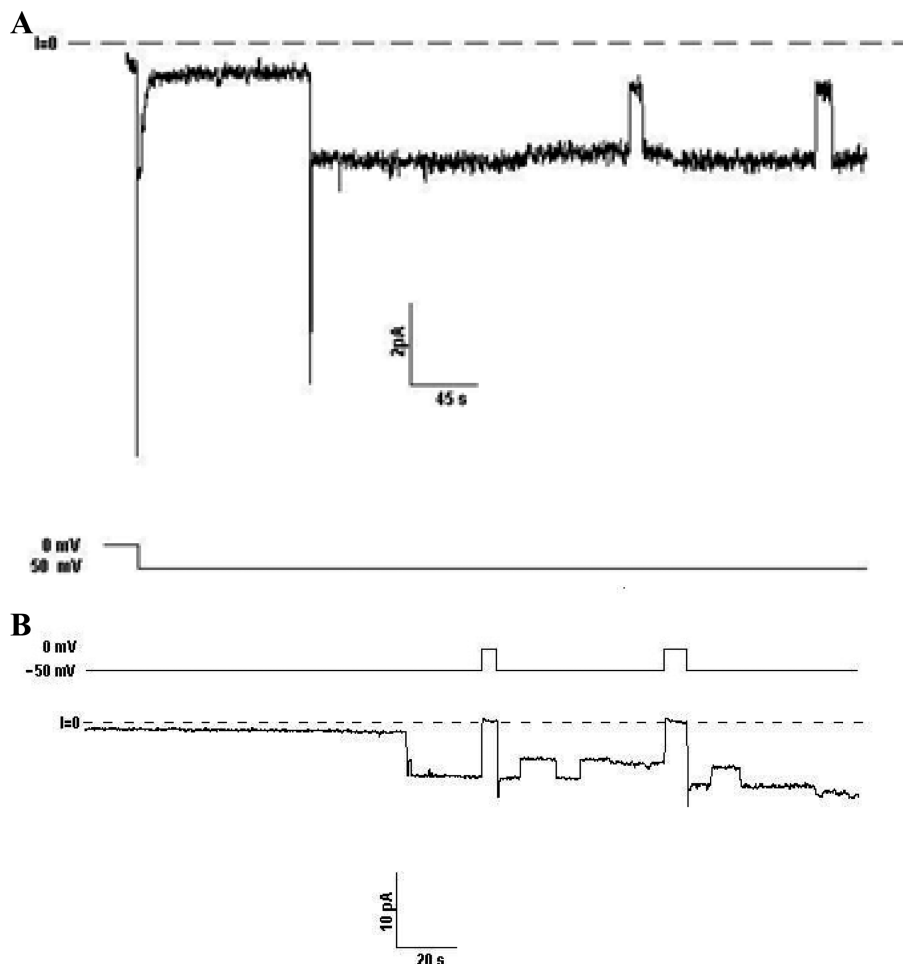


Figure 2. Heterodisperse single channel conductances of tau channels. (A) Single channel conductance of 35 pS. (B) Heterogeneous conductances of 121, 35, and 53 pS recorded, suggesting multiple subunits continue to form channel structures with different single channel conductances.

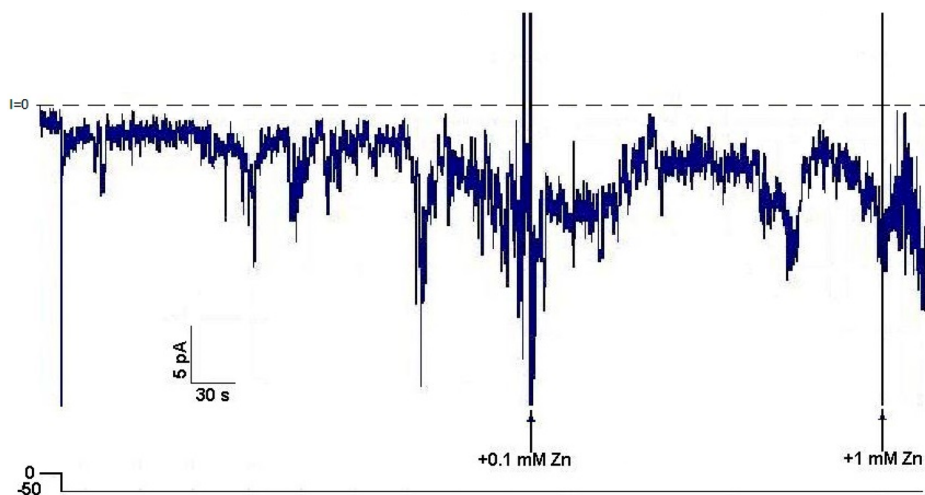


Figure 3. Unlike other amyloid beta channels, tau channels are not blocked by Zn^{2+} ions even at higher concentrations (1 mM).

conductance shown were not unique as multiple single channel conductance transitions were observed (35, 53, and 121 pS), a phenomenon common to the channel forming amyloid peptides. This may reflect aggregation of tau monomers into aggregates of varying number, size, and single channel conductance. Current induced only at acidic pH 5.2, there was no activity registered at pH 7.4. A reversal potential of 7 to

11 mV was recorded in a 10-fold gradient of NaCl, indicating little ion selectivity between negatively and positively charged ions. However, tau channels are not blocked by Zn^{2+} ions (Figure 3) unlike channels formed by other amyloid peptides, even at higher concentrations of Zn^{2+} (1 mM). Channels could last for several hours in the membrane indicating that they were irreversibly associated with the membrane.

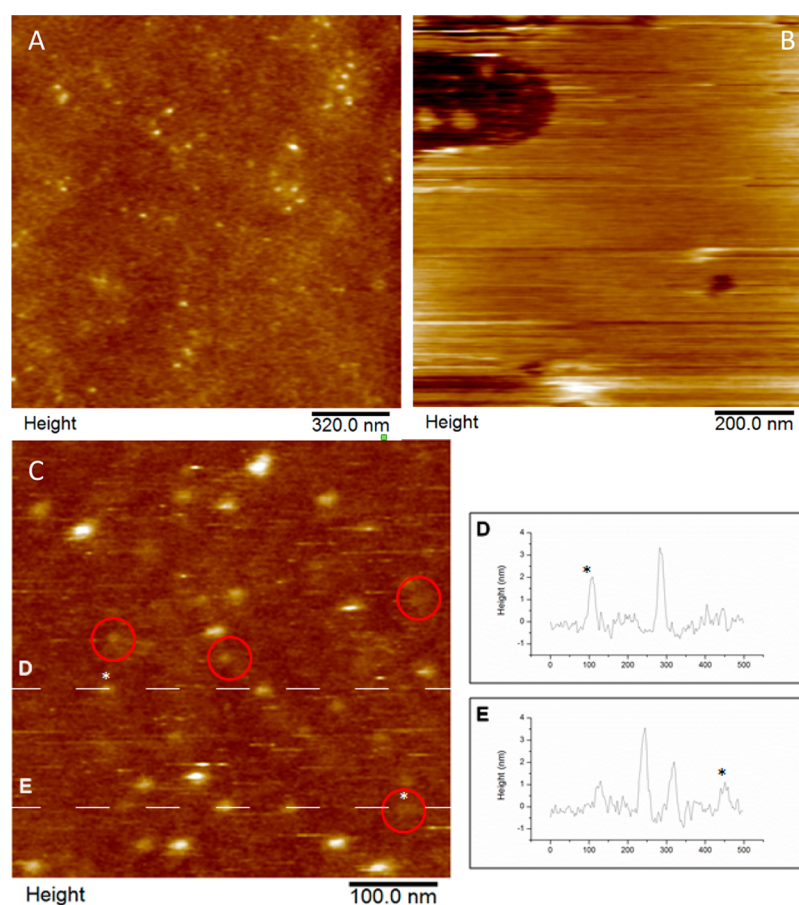


Figure 4. (A) AFM images of tau protein on mica reveal monomers and oligomers distributed on the surface. (Height scale is 2 nm). (B) DOPS bilayers in the absence of tau protein reveal uniformly smooth bilayers with little deviation in the surface height. (C) Tau oligomers on DOPS lipid bilayer. Tau predominantly forms globular oligomers on the bilayer surface. Few porelike structures are observed within the bilayer (red circles). (D, E) Plots of line traces indicated in the AFM image intersect a globular oligomer (D) and porelike structure (E) (indicated by asterisks).

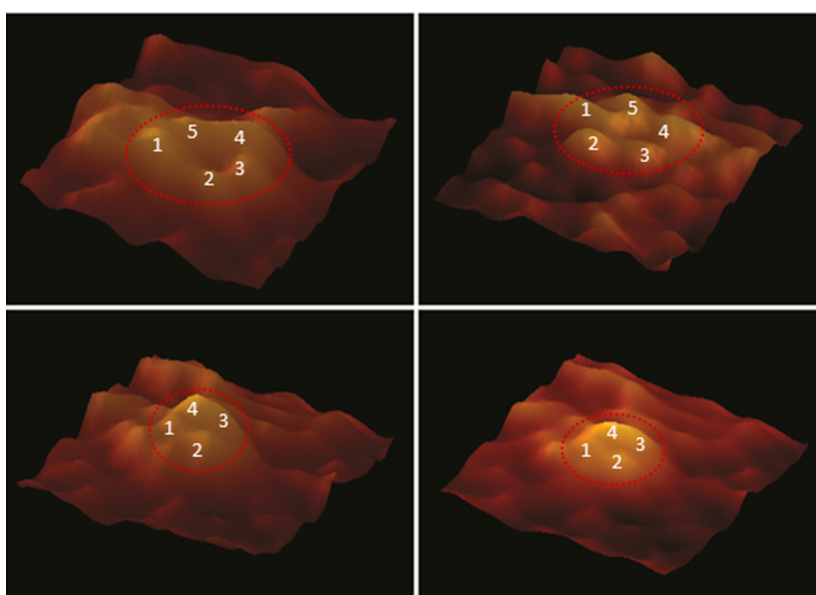


Figure 5. Three-dimensional representation of porelike structures of tau protein in a lipid bilayer.

Tau Forms Porelike Structures. Tau protein predominantly forms globular oligomers in the presence of a negatively charged surface. Contact mode AFM images of tau on mica (Figure 4A) show a population of tau monomers and

oligomers. The diameter and height of tau proteins on mica is $28.6 (\pm 6.4)$ and $1.21 (\pm 0.44)$ nm, respectively. We investigated the structure of aggregated tau in reconstituted into lipid membranes. Tapping mode AFM images reveal that

tau predominantly forms globular oligomers on DOPS planar bilayers (Figure 4C).

High-resolution AFM imaging of tau interacting with lipid bilayers reveals the presence of porelike structures within the bilayer (Figure 5). Porelike structures were identified by the presence of subunits forming a circular group with a small height increase relative to the bilayer surface.²⁵ Observed structures were heterogeneous in size and shape. The protrusion height of these structures is approximately 1.5–1.8 nm from the surface of the surrounding bilayer. The inner diameter of these structures is approximately 2.0–3.9 nm, and average outer diameter is 15.1–20.5 nm. For comparison, the globular oligomers of tau have an average height of 4.33 (± 1.68 nm) and an average diameter of 14.6 (± 4.4 nm). The three-dimensional structures of observed porelike structures reveal four to five subunits circularly arranged around a central channel. Few observed structures have a distinct central depression, likely due to features being obscured by the AFM tip morphology and local movements of subunits within the lipid bilayer.

The Porelike Structures Are Distinct from Annular Protofibrils of Tau. The structure of the porelike structures formed by tau is distinct from the annular protofibrils as revealed by AFM imaging (data not shown). The average height of annular protofibrils is 3.60 ± 0.77 nm, which is comparable to that of globular oligomers of tau and distinctly greater than that of the porelike structures. The average inner and outer diameter of an annular protofibril is 16.5 (± 9.6) nm and 52.9 (± 23.3) nm, respectively.

DISCUSSION

Both electrical conductance and high resolution AFM imaging demonstrate that tau forms conducting ion channels in planar lipid bilayers. The data is remarkably similar to that previously reported for other amyloid channels. Specifically, tau channels are irreversibly associated with the membrane, heterodisperse, that is, exhibiting multiple single channels conductances and relatively nonselective for common physiologic ions. These channels differ from amyloid channels in at least 2 properties. First, tau channels require acidic pH for formation. This may be explained by increased aggregation of tau at acidic pH and may reflect the kinetics of the aggregation process. Further experiments are needed to elucidate this. Second, tau channels are not blocked by Zn^{2+} . This might reflect a difference in the structure of tau channels from other amyloid peptides. The tau protein (2N4R) that we used is far larger (MW 45 900) than most of the amyloid peptides shown to form channels. The sheer bulk of the protein may be responsible for the lack of Zn^{2+} block. Again, further investigations are required to understand this.

The channels observed in our experiments are due to tau. Control experiments without tau showed no changes in membrane conductance. Experiments with tau at physiologic pH also showed no channels, but when the pH was lowered sufficiently, channels formed. Raising the pH after channels formation did not cause the channels to close or disappear, indicating that acidic pH is likely required for tau aggregation and/or membrane insertion. Channels were stable structures with long lifetimes and relative permanence in the membrane. This argues strongly for these channels representing a tau protein mediated, rather than a lipid defect.

The multiplicity of single channel conductances observed in the tau channels suggests that multiple species of tau oligomers

are forming channels. This is characteristic of channel forming molecules that aggregate. Alternately, the multiple conductance levels observed could be conformations of a single (or several) molecular entities. Further work is required to resolve this issue.

Furthermore, the multiplicity of channel conductance is supported by AFM results (Figure 5). The observed channel size is smaller than expected considering the molecular weight of tau compared to that of other amyloids.^{20,25,26} The size similarity of tau and other amyloid structures can be explained by a greater fraction of aggregated tau being embedded within the lipid bilayer. For comparison, connexin hemichannels, acetylcholine receptor channels (AChR), Na and K channels, for example, all with similar molecular weights, are only 8–12 nm in outer diameter with an ionic pore of 1–3 nm.^{27–29}

The tau channels reported here could play a role in the pathophysiology of Alzheimer's and other dementias. These channels could depolarize membranes, disrupt Ca^{2+} regulation, depolarize mitochondria, and deplete cellular energy stores. These effects could impair synaptic function including long-term potentiation and lead to the deficits in memory that are characteristic of Alzheimer's and other neurodegenerative diseases. Tau channels might also interact with amyloid beta channels. There is strong evidence for a role of amyloid beta channels in Alzheimer's pathophysiology. Tau channels could exacerbate the effects of amyloid beta channels. This notion is supported by the results with transgenic mice which show that mice containing amyloid beta and tau develop more severe memory problems at an earlier stage than mice with either peptide alone.³⁰

Our past results do not address possible interactions of tau and amyloid beta. There is evidence that tau is present in amyloid plaques and that amyloid beta and tau may bind when in the appropriate β sheet conformation. This amyloid "synergy" may lead to new pathways for drug development to arrest the relentless progression of these devastating neurological diseases. Blockers of amyloid beta channels can prevent neurotoxicity in vitro. Future investigations of tau channels should search for protective blockers that might be therapeutic lead compounds. The multiplicity of single channels conductances observed in the tau channels suggests that multiple species of tau oligomers are forming channels. This phenomenon has been observed with almost all other amyloid channels and reflects the necessity of aggregation/dysregulation as a prerequisite for channel formation. The heterodisperse nature of channels may underlie the failure of anti-amyloid drugs to help patients with Alzheimer's disease. If many species are toxic, a single drug may be insufficient to stop the disease process as it only targets one species out of many.

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Funding

This research was supported by the National Institutes of Health (National Institute on Aging Grant AG028709 to R.L.).

Notes

The authors declare no competing financial interest.

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