Cellular Prion Protein Mediates the Toxicity of β-Amyloid Oligomers

Implications for Alzheimer Disease

Haakon B. Nygaard, MD; Stephen M. Strittmatter, MD, PhD

Alzheimer disease (AD) is the most common cause of age-related dementia, affecting more than 25 million people worldwide. The accumulation of insoluble β-amyloid (Aβ) plaques in the brain has long been considered central to the pathogenesis of AD. However, recent evidence suggests that soluble oligomeric assemblies of Aβ may be of greater importance. β-Amyloid oligomers have been found to be potent synaptotoxins, but the mechanism by which they exert their action has remained elusive. Herein, we review the recently published finding that cellular prion protein (PrPc) is a high-affinity receptor for Aβ oligomers, mediating their toxic effects on synaptic plasticity. We further discuss the relationship between AD and PrPc and the potential clinical implications. Cellular prion protein may provide a novel target for therapeutic intervention in AD.

Arch Neurol. 2009;66(11):1325-1328

Alzheimer disease (AD) is a debilitating neurodegenerative disorder, estimated to affect at least 26.6 million people worldwide.1 Over the next 40 years, this number is predicted to increase 4-fold,1 stressing the need for more effective therapeutics. For many years, the leading hypothesis for the pathogenesis of AD has involved the toxic effects of a gradual buildup of β-amyloid (Aβ) plaques in the brain.2 β-Amyloid is produced after sequential processing of the amyloid precursor protein (APP),2 and increased levels of the Aβ1-42 species are thought to be a key early event in AD.3 A major criticism of the amyloid hypothesis has been that the burden of fibrillar Aβ, the insoluble species constituting plaques, correlates poorly with the degree of dementia4 and brain atrophy,5 and cognitively normal individuals may have significant plaque deposition at autopsy.6 However, more recent findings have elucidated different assemblies of Aβ with varying degrees of toxic effects. β-Amyloid can exist as soluble monomers and oligomers, intermediate protofibrils, and insoluble fibrillar aggregates.7 Of these, soluble Aβ oligomers are potent synaptotoxins,8 and their concentration correlates with disease severity.9

Oligomers are small peptide polymers, and various toxic Aβ oligomers have been reported, ranging from dimers and trimers10 to 100mers.11 Nanomolar concentrations of Aβ oligomers strongly inhibit long-term potentiation (LTP),12,13 a leading experimental model for the synaptic changes underlying learning and memory. Recent evidence also suggests that synapse degeneration, likely central to the memory impairment in AD,14 first involves the single synaptic contact points between axons and dendrites found at dendritic spines.15 Consistent with their role in neurodegeneration, Aβ oligomers cause significant decrease in dendritic spine density in cultured hippocampal neurons.15 Behavioral assessments in rodents have confirmed these findings, complementing a growing number of in vitro investigations. β-Amyloid oligomers greatly impair spatial memory when administered to otherwise healthy rats.12,16

The concept of oligomeric Aβ synaptotoxins has helped strengthen the amyloid hypothesis. In transgenic mice, lev-
els of specific Aβ oligomers closely match deficits in spatial memory, and clinically the amount of soluble Aβ in the brain correlates well with the degree of dementia. Moreover, Aβ oligomers isolated directly from human AD brains have been found to be equally toxic as synthetically derived Aβ. Despite these recent breakthroughs, the mechanisms by which Aβ oligomers exert their action have not been well understood. The effects on neurons are rapid and specific, and together the accumulated data have suggested the presence of a high-affinity Aβ oligomer receptor. This putative receptor would likely be central to the pathogenesis of AD and provide an attractive target for therapeutic intervention. Herein, we review the recent finding that cellular prion protein (PrPc) is a high-affinity binding site for Aβ oligomers, mediating their potent inhibitory effects on synaptic plasticity. We further discuss the association between PrPc and AD and the potential clinical relevance of these findings.

EXPRESSION CLONING IDENTIFIES PRPC AS A HIGH-AFFINITY BINDING SITE FOR Aβ OLIGOMERS

To detect Aβ oligomer binding sites, a biotin-tagged Aβ1-42 peptide was first synthesized. Biotin is frequently used as a molecular marker because of its extraordinarily high affinity for the protein avidin that, in turn, can be used for detection. The Aβ was oligomerized and applied to COS-7 cells expressing different complementary DNA (cDNA) obtained from an adult mouse brain library. At baseline, COS-7 cells show minimal binding of Aβ oligomers compared with hippocampal neurons, making these cells ideal for this type of screen. Biotin-Aβ oligomers potently bind hippocampal neurons, whereas monomeric Aβ does not, suggesting a necessary conformation of Aβ conferred by oligomerization.

Of 225,000 clones from the adult mouse cDNA library screened in the assay, 2 were found to bind biotin-Aβ oligomers. Both of these individual clones were subsequently shown to encode for full-length mouse PrPc. In addition to this unbiased genome-wide screen, a more specific library of known transmembrane proteins at lower stringency was assessed. As summarized in the Table, some were found to exhibit Aβ oligomer binding but with lesser affinity and selectivity than for PrPc. Several putative receptors for Aβ have previously been reported, including the receptor for advanced glycation end products, α7 nicotinic acetylcholine receptor, and tumor necrosis factor receptor 1. In the binding assay reviewed herein, only PrPc had high affinity and high specificity for Aβ oligomers.

Aβ OLIGOMERS BIND TO A SPECIFIC REGION OF PRPC

Cellular prion protein is a glycosylphosphatidylinositol-linked extracellular membrane protein of approximately 250 amino acids that is abundantly expressed in the nervous system. It is likely best known to clinicians because of its role in human prion disease. Using PrPc deletion mutants, the exact binding site of Aβ oligomers was mapped to the region of amino acids 95 to 110. This area is within the unstructured central domain of PrPc (amino acids 95-134), which has been implicated in neuronal toxic effects in vitro and in extensive neurodegeneration in mice. α-Secrease, which cleaves APP within the Aβ domain (precluding the toxic Aβ peptide), also cleaves PrPc within its unstructured central domain at amino acids 110 to 111. Therefore, α-secretase may act to counter neurodegeneration by preventing the production of Aβ and by keeping potentially detrimental regions of PrPc in check. It is unknown whether cleavage of PrPc by α-secretase also affects Aβ oligomer binding.

PRPC MEDIATES INHIBITION OF LTP BY Aβ OLIGOMERS

To evaluate the functional role of the Aβ oligomer–X-PrP interaction, the effects of Aβ oligomers on LTP were measured in brain sections from mice in which the PrPc gene was knocked out (PRNP−/−) (OMIM 176640). In wild-type hippocampal sections, a brief train of pulses in the theta frequency to Schaffer collateral fibers results in an increase in the excitatory postsynaptic potentials that can last for hours. This change in synaptic strength in response to a specific stimulus is believed to be a fundamental mechanism underlying learning and memory. When nanomolar concentrations of Aβ oligomers were applied to wild-type sections, LTP was strongly inhibited, as has been reported by numerous laboratories. In contrast, no inhibitory effects on LTP were seen when the same Aβ preparation was applied to brain sections from PRNP−/− animals, suggesting that Aβ oligomer signaling is mediated through PrPc. To confirm these findings, wild-type brain sections were pre-treated with 6D11, a specific antibody to PrPc found to block Aβ oligomer binding. When these pre-treated sections were exposed to the Aβ solution, LTP was unaffected.

### Table. β-Amyloid (Aβ) Oligomer Protein Binding

<table>
<thead>
<tr>
<th>Cell Surface Protein</th>
<th>Aβ Oligomer Binding</th>
<th>Oligomer Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrPc</td>
<td>++ + +</td>
<td>++ + +</td>
</tr>
<tr>
<td>APLP1</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>TMEM30A</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>TMEM30B</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>RAGE</td>
<td>++</td>
<td>?</td>
</tr>
<tr>
<td>APP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Doppel</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SPRN</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α7 Nicotinic acetylcholine receptor</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>APLP2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: APLP, amyloid precursor–like protein; APP, amyloid precursor protein; PrPc, cellular prion protein; RAGE, receptor for advanced glycation end products; SPRN, shadow of prion protein; TMEM, transmembrane protein; +, slight; + +, low; + + + +, high; ?, unknown.

*Refers to the preferential binding of oligomeric Aβ over freshly prepared Aβ.*
further suggesting a critical role of PrP\(^\text{a}\) in A\(\beta\) oligomer–induced synaptotoxic effects.

Because PrP\(^\text{a}\) is an extracellular protein, downstream A\(\beta\) oligomer signaling is likely mediated via a putative PrP\(^\text{a}\)-associated transmembrane coreceptor (Figure). This putative coreceptor may have a critical role in AD-related neurodegeneration and, once identified, could provide an additional potential therapeutic target.

**PRION PROTEIN AND AD**

For at least a decade, an indirect association between AD and PrP\(^\text{a}\) has been lingering in the literature. Early interest was fueled mostly by the fact that AD and human prion disease involve aggregates of pathologic proteins in the brain\(^{25}\) and that the amyloid precursor–like protein was reported to bind PrP\(^\text{a}\).\(^{26}\) Recently, more specific evidence has emerged linking PrP\(^\text{a}\) to AD. From a genetic perspective, interest has been growing in the \(PRNP\) methionine/valine polymorphism (M129V) and its relationship to sporadic AD. Homozygosity at this codon, which is located within the unstructured central domain of PrP\(^\text{a}\), is associated with an increased risk of sporadic Creutzfeldt-Jakob disease.\(^{27}\) Others have found that this genotype confers an increased risk for sporadic AD in certain populations.\(^{28}\) Whether polymorphisms within the \(PRNP\) gene may affect A\(\beta\) oligomer binding to PrP\(^\text{a}\) and alter susceptibility to AD is unknown, but it remains an intriguing possibility.

Cellular prion protein has also been reported to regulate APP processing by modulating \(\beta\)-secretase (BACE1) activity, but the results have been conflicting. Cells coexpressing APP and PrP\(^\text{a}\) have a significant reduction in secreted A\(\beta\) compared with cells expressing APP alone.\(^{29}\) The region of PrP\(^\text{a}\) responsible for this action was found to be in the N-terminus (amino acids 23-26), as no effects on BACE1 or A\(\beta\) levels were seen when this segment was removed.\(^{29}\) However, overexpressing PrP\(^\text{a}\) in transgenic APP mice caused a modest increase of A\(\beta\) plaques in the brain,\(^{30}\) in contrast to the lowering effect of PrP\(^\text{a}\) found in vitro. It is unclear whether binding of A\(\beta\) oligomers to PrP\(^\text{a}\) might affect its proposed ability to regulate BACE1, setting up a potential feedback loop in response to A\(\beta\) levels, and this is an issue worth investigating further as the field moves forward.

**CONCLUSIONS**

In summary, we have reviewed the recent finding that PrP\(^\text{a}\) functions as a high-affinity receptor to mediate the deleterious effects of A\(\beta\) oligomers on synaptic plasticity. Although much work remains to assess whether targeting PrP\(^\text{a}\) or its putative coreceptor can be effective treatments in human AD, the potential is readily apparent. Cellular prion protein represents the first possible target in AD directly disrupting the signaling pathway of A\(\beta\) and might provide a more functional approach to relieving AD symptoms. Most important, modulating PrP\(^\text{a}\) function does not seem to produce serious adverse effects, as \(PRNP\) knockout mice have minimal phenotypic changes and wild-type animals treated with PrP\(^\text{a}\) antibodies do well. A large body of work on PrP\(^\text{a}\) already exists because of its role in prion diseases. This knowledge could greatly facilitate drug discovery and our understanding of the downstream mechanisms of the A\(\beta\) oligomer–X-PrP\(^\text{a}\) interaction.

Accepted for Publication: May 13, 2009.

Correspondence: Stephen M. Strittmatter, MD, PhD, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, 295 Congress Ave, Boyer Center for Molecular Medicine 436, New Haven, CT 06536-0812 (stephen.strittmatter@yale.edu).

Author Contributions: Study concept and design: Nygaard and Strittmatter. Acquisition of data: Nygaard and Strittmatter. Analysis and interpretation of data: Nygaard and Strittmatter. Drafting of the manuscript: Nygaard and Strittmatter. Critical revision of the manuscript for important intellectual content: Nygaard and Strittmatter. Obtained funding: Strittmatter. Administrative, technical, and material support: Strittmatter. Study supervision: Strittmatter.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grants from the Cure Alzheimer’s Fund, National Institutes of Health, and Falk Medical Research Trust (Dr Strittmatter).

REFERENCES

3. Younkin SG. The role of Aβ/β-H9252
8. Lambert MP, Barlow AK, Chromy BA, et al. Dif-
7. Klein WL. Aβ/β-H9252
secreted oligomers of amyloid β-protein poten-
tially inhibit hippocampal long-term poten-
13. Wang HW, Pasternak JF, Kuo H, et al. Soluble oligo-
mers of β-amyloid (1-42) inhibit long-term po-
tention but not long-term depression in rat den-
tate gyrus. Brain Res. 2002;924(2):133-140.
15. Lacor PN, Buniel MC, Furlow PW, et al. Aβ oligo-
mer–induced aberrations in synapse composi-
tion, shape, and density provide a molecular ba-
sis for loss of connectivity in Alzheimer’s disease.
16. Lesn S, Koh MT, Koltinek L, et al. A specific amy-
loid-β-protein assembly in the brain impairs memory.
17. Shankar GM, Li S, Mehta TH, et al. Amyloid-β-pro-
tein dimers isolated directly from Alzheimer’s brains impair synaptic plasticity and memory. Nat Med.
2006;14(8):837-842.
18. Yan SD, Chen X, Fu J, et al. RAGE and amyloid-β
1996;382(6593):685-691.
19. Wang HY, Lee DH, D’Andrea MR, Peterson PA,
Shank RP, Reitz AB. β-Amyloid, β binds to α7 nico-
tinic acetylcholine receptor with high affinity: im-
plications for Alzheimer’s disease pathology. J Biol
inhibition of long-term potentiation is mediated
21. Aguzzi A, Baumann F, Bremer J. The prion’s elu-
sive reason for being. Annu Rev Neurosci. 2008;
31:439-477.
22. Forloni G, Angeretti N, Chiesa R, et al. Neurotox-
362(6420):543-546.
23. Baumann F, Telson M, Brabecck, et al. Lethal re-
cessive myelin toxicity of prion protein lacking its
MV, Checler F. Regulation of [APP and PrP cleav-
age by α-secretase: mechanistic and therapeutic
202-211.
25. Aguzzi A, Haass C. Games played by rogue pro-
teins in prion disorders and Alzheimer’s disease.
Identification of candidate proteins binding to prion
27. Palmer MS, Dryden AJ, Hughes JT, Collinge J.
Homozogous prion protein gene predominate to
sporadic Creutzfeldt-Jakob disease [published cor-
correction appears in Nature. 1991;352(6335):547].
of PRNP gene associated with Alzheimer’s dis-
esease? a case-control study and a meta-
analysis. Neurobiol Aging. 2006;27(5):770.e1-
770.e5. http://www.journals.elsevierhealth.com
/periodicals/nba/article/PiIS0187458005001612/
protein regulates β-secretase cleavage of the
Alzheimer’s amyloid precursor protein. Proc Natl
30. Schwarz-Ecker K, Keyvan K, Görtz N, West-
away D, Sachser N, Paulus W. Prion protein (PrPc)
promotes β-amyloid plaque formation. Neurobiol

Announcement

Sign Up for Alerts—It's Free! Archives of Neurology of-
fers the ability to automatically receive the table of con-
ents of Archives when it is published online. This also
allows you to link to individual articles and view the ab-
tract. It makes keeping up-to-date even easier! Go to http://pubs.ama-assn.org/misc/alerts.dtl to sign up for this
free service.

©2009 American Medical Association. All rights reserved.