



Breaking the Cycle, Cholesterol Cycling, and Synapse Damage in Response to Amyloid- β



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ABSTRACT: Soluble amyloid- β ($A\beta$) oligomers, a key driver of pathogenesis in Alzheimer disease, bind to cellular prion proteins (PrP^C) expressed on synaptosomes resulting in increased cholesterol concentrations, movement of cytoplasmic phospholipase A₂ (cPLA₂) to lipid rafts and activation of cPLA₂. The formation of $A\beta$ -PrP^C-cPLA₂ complexes was controlled by the cholesterol ester cycle. Thus, $A\beta$ activated cholesterol ester hydrolases which released cholesterol from stores of cholesterol esters; the increased cholesterol concentrations stabilised $A\beta$ -PrP^C-cPLA₂ complexes. Conversely, cholesterol esterification reduced cholesterol concentrations causing the dispersal of $A\beta$ -PrP^C-cPLA₂. In cultured neurons, the cholesterol ester cycle regulated $A\beta$ -induced synapse damage; inhibition of cholesterol ester hydrolases protected neurons, whereas inhibition of cholesterol esterification increased the $A\beta$ -induced synapse damage. Here, I speculate that a failure to deactivate signalling pathways can lead to pathology. Consequently, the esterification of cholesterol is a key factor in the dispersal of $A\beta$ -induced signalling platforms and synapse degeneration.

KEYWORDS: Cholesterol, amyloid, cholesterol ester, phospholipase A2, synapse

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Commentary

A recent paper from my laboratory highlighted the involvement of the cholesterol ester cycle in response to amyloid- β ($A\beta$) oligomers, a key driver of pathogenesis in Alzheimer disease (AD).¹ The study concentrated on events occurring at 2 levels. At a cellular level, we demonstrated that manipulation of the cholesterol ester cycle affected $A\beta$ -induced synapse damage. The biochemistry and cell signalling associated with $A\beta$ was also examined in isolated synaptosomes. To our knowledge, this was the first study to show that changes in membrane cholesterol concentrations mediated by the cholesterol ester cycle significantly affect a major cell signalling pathway.

Because cholesterol is such a key molecule involved in the regulation of membrane structure and function, it is not surprising that disturbances in cholesterol homeostasis are associated with neurodegenerative diseases² and more specifically with the pathogenesis of AD, as reviewed by Chang et al.³ The amyloid hypothesis, the prevailing theory explaining the pathogenesis of AD, states that the accumulation of $A\beta$ peptides within the brain is responsible.⁴ Although several studies have reported that cholesterol or cholesterol-binding proteins affect $A\beta$ production, our study examined the role of cholesterol on the toxic effects of $A\beta$; primarily its effects on synapse degeneration as the loss of synapses and synaptic proteins shows a close correlation with the severity of dementia.⁵

The initial and key observation was that the addition of $A\beta$ increased synaptic cholesterol concentrations, an observation that is consistent with reports of increased cholesterol concentrations in $A\beta$ -positive synapses in the cortex of patients with AD.⁶ Surprisingly, this was not due to cholesterol synthesis, rather the

$A\beta$ -induced increase in synaptic cholesterol concentrations was controlled by the cholesterol ester cycle; it was accompanied by a corresponding reduction in cholesterol ester concentrations indicating the activation of a cholesterol ester hydrolase (CEH). Furthermore, selective CEH inhibitors blocked the $A\beta$ -induced increase in synaptic cholesterol concentrations.

Cholesterol is highly enriched in synaptic membranes, and given that cholesterol concentrations in cell membranes are critical for the formation of signalling platforms in lipid rafts,⁷ we argued that fluctuations in cholesterol concentrations could alter the functions of lipid rafts. Lipid raft formation is associated with the aggregation of the cellular prion protein (PrP^C), identified as a receptor for $A\beta$,⁸ by $A\beta$ oligomers.⁹ Notably, the increase in synaptic cholesterol concentrations was associated with the toxic $A\beta$ oligomers^{10,11} rather than non-toxic $A\beta$ monomers.¹² Here, we speculate that $A\beta$ oligomers, but not monomers have the ability to cross-link cellular receptors, a hypothesis consistent with observations that synaptic abnormalities are caused by the cross-linkage of PrP^C with monoclonal antibodies.¹³ Cellular prion protein acts as a scaffold protein that organises signalling complexes and in neurons the clustering of specific glycosylphosphatidylinositols attached to PrP^C caused aberrant cell signalling and synapse degeneration.¹⁴

Cellular prion protein is associated with numerous cell signalling pathways including cytoplasmic phospholipase A₂ (cPLA₂)¹⁵ which leads to the production of platelet-activating factor (PAF) and prostaglandins. The observations that concentrations of prostaglandin E₂ (PGE₂) and PAF are raised in the brains of patients with AD when compared with non-demented



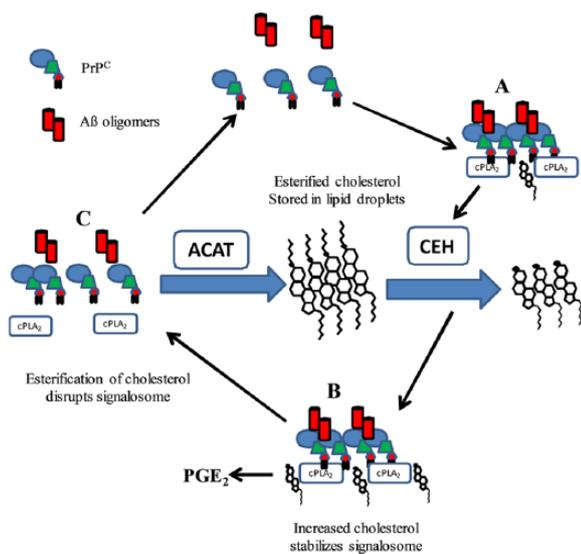


Figure 1. Putative control of synaptic signalosome created by A β oligomers: The binding of A β oligomers cross-links PrP^C within lipid rafts (A) recruits and activates cPLA₂. Subsequently, CEHs are activated causing the release of cholesterol into the membrane; (B) the increase in cholesterol stabilises signalling complexes. cPLA₂ is activated within the signalling platform releasing PGE₂ which at high concentrations causes synapse degeneration. (C) ACAT activity resulted in the esterification of cholesterol, reduced free cholesterol concentrations in the membrane, and caused the dispersal of lipid raft signalling complexes. A β indicates amyloid- β ; ACAT, acetyl-coenzyme A acetyltransferase; cPLA₂, cytoplasmic phospholipase A₂; PGE₂, prostaglandin E₂; PrP^C, cellular prion proteins.

controls^{16,17} suggest that aberrant activation of cPLA₂ is associated with synapse degeneration and clinical symptoms.

We hypothesised that A β oligomers cross-linked PrP^C leading to the activation of CEHs and increased cholesterol concentrations; these stabilise a signalling platform that included activated cPLA₂ and led to increased production of PGE₂ (Figure 1). This hypothesis was supported by the close correlations between the concentrations of cholesterol, raft-resident cPLA₂, activated cPLA₂, and PGE₂ production following the addition of A β . Furthermore, pre-treatment with CEH inhibitors prevented the formation of A β -PrP^C complexes, the A β -induced increase in cholesterol, the movement of cPLA₂ to lipid rafts, the activation of cPLA₂, and the production of PGE₂. When tested on cultured neurons, CEH inhibitors reduced the A β -induced synapse damage indicating that these enzymes are critical for A β toxicity. This suggests that the events measured within synaptosomes relate to the process of A β -induced synapse degeneration.

Time course studies demonstrated that the A β -induced increase in cholesterol/reduction in cholesterol esters, PrP^C-A β complexes, and the amounts of cPLA₂ within lipid rafts were all transient. Notably, the return of cholesterol/cholesterol ester concentrations to basal levels following esterification was closely associated with the dissociation of A β -PrP^C complexes and the return of cPLA₂ to the cytoplasm. In addition, pre-treatment

with selective inhibitors of acetyl-coenzyme A acetyltransferase (ACAT), an enzyme that esterifies cholesterol, resulted in increased A β -PrP^C complexes, higher cholesterol concentrations, increased time that cPLA₂ spent within lipid rafts, increased activation of cPLA₂, and higher PGE₂ concentrations.

Conditions in which signalling platforms fail to dissociate may lead to sustained activation of signalling pathways and lead to cell disruption and disease. Consequently, the dissociation of signalling platforms is thought to be an important physiological process that limits the intensity of cell signalling. An altered cholesterol ester cycle resulting in accumulation of cholesterol esters has been reported in patients with AD.¹⁸ In this Commentary, we speculate that high concentrations of A β could 'break the cycle' by reducing the esterification of cholesterol and consequently preventing the dissociation of signalling platforms. In this respect, it was noteworthy that inhibition of ACAT in neuronal cultures significantly increased the A β -induced synapse damage. Acetyl-coenzyme A acetyltransferases may affect different aspects of AD pathogenesis. For example, ACAT inhibitors have been proposed as treatments for AD because they reduced A β production in studies where they were used throughout the course of an experimental disease.¹⁹ However, this study shows that ACAT inhibitors can increase synapse damage in the presence of A β . Consequently, ACAT inhibitors might be able to prevent the development of AD but maybe contraindicated in the latter stages of AD where concentrations of A β are already raised.

In summary, our article demonstrated the role of the cholesterol ester cycle in A β -induced cell signalling at synapses and synapse degeneration. The release of cholesterol stabilises the complexes formed between PrP^C and A β that activate cPLA₂. Conversely, the esterification of cholesterol facilitates the dissociation of PrP^C-A β complexes and deactivation of cPLA₂.

Author Contributions

CB reviewed and approved the final manuscript.

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