PERSPECTIVE

Enhanced neuronal degradation of amyloid-β oligomers allows synapse regeneration



Neurodegeneration in AD is preceded by the intraneuronal accumulation of A β (Takahashi et al., 2002). The chronic nature of AD suggests that it is the slow, progressive accumulation of A β that triggers neurodegeneration and hence the clinical symptoms. As neural regeneration cannot occur in the presence of toxic concentrations of A β , the first step in regeneration must be the removal of toxic A β . The concentration of A β in neurons is dependent upon both the rate of A β production and also the rate of A β degradation. While there are numerous studies examining A β production, the capacity of neurons to degrade A β once it has been formed has received little attention. A recent publication from my laboratory explored the hypothesis that the slow clearance of A β from neurons is a significant factor in the accumulation of A β within the brain that leads to synapse damage and dementia in AD (Simmons et al., 2014).

Soluble Aß oligomers are found predominantly within detergent-resistant, cholesterol-dense membrane micro-domains which are referred to as lipid rafts (Lee et al., 1998; Kawarabayashi et al., 2004; Williamson et al., 2008). The targeting of $A\beta$ to rafts may have important biological consequences, as many raft-associated proteins traffic through cells via "recycling pathways" which avoid the lysosomes (Nichols et al., 2001) (organelles responsible for protein degradation). In cultured neurons, Aβ oligomers behaved like a classic "raft protein"; they were not found within lysosomes and consequently were cleared slowly from neurons, with a half-life of greater than 5 days. Thus the targeting of AB oligomers to rafts may contribute to their gradual accumulation within neurons and subsequently their toxic effects. We postulated that the rate of AB degradation within neurons could be increased following pharmacological manipulation.

Cholesterol has multiple effects upon cell membranes and is instrumental in regulating membrane fluidity, protein trafficking, endocytosis and exocytosis (Nichols et al., 2001). Critically, the concentrations of cholesterol within cell membranes affect the formation and function of lipid rafts (Pike, 2004; Rajendran and Simons, 2005; Hancock, 2006). Cholesterol synthesis inhibitors that caused a mild reduction in cholesterol concentrations altered the fate of A β oligomers in cultured neurons (Simmons et al., 2014). Although cholesterol depletion did not affect the binding of A β oligomers to neurons, significantly less A β was found within lipid rafts. Greater amounts of A β were found within lysosomes and in cholesterol-depleted neurons the halflife of A β was reduced to less than 24 hours. Such results indicate that the pharmacological manipulation of neurons can significantly increase the clearance of A β .

As cholesterol is required for normal cell function cholesterol synthesis inhibitors can have multiple unwanted effects. Treatment with inhibitors of cytosolic phospholipases A_2 (cPLA₂), an enzyme which affects intracellular trafficking (de Figueiredo et al., 1998; Grimmer et al., 2005), or platelet-activating factor (PAF) antagonists had the same effects on $A\beta$ metabolism in neurons as cholesterol depletion. These drugs reduced the activation of cholesterol ester hydrolases (CEH), enzymes that constitute a part of the cholesterol cycle which regulates the concentrations of cholesterol in cell membranes (Chang et al., 2006). CEHs release free cholesterol from the stores of biologically inert cholesterol esters. A β oligomers activate cPLA₂ (Bate and Williams, 2011; Desbene et al., 2012) resulting in activation of CEH and increased cholesterol concentrations within specific membranes, without affecting total cellular cholesterol concentrations (Simmons et al., 2014). Critically, inhibitors of CEH had a similar effect to cholesterol synthesis inhibitors on the trafficking of $A\beta$ in neurons. They reduced concentrations of A β in lipid rafts, increased A β in lysosomes, and reduced the half-life of $A\beta$ to less than 24 hours.

Physiologically relevant (low nanomolar) concentrations of $A\beta$ oligometrs, similar to those found in the cerebrospinal fluid of AD patients (Lue et al., 1999; McLean et al., 1999; Bibl et al., 2006; Mc Donald et al., 2010), were incubated with cultured mouse cortical neurons. This concentration of A^β oligomers did not affect neuronal survival but did cause significant synapse degeneration, as measured by the loss of synaptic proteins including synaptophysin and cysteine string protein. It is worth noting that in neuronal cultures synapse damage occurs at concentrations approximately 100-fold less than the concentrations required to cause neuronal death. Thus, these conditions mimic the early stages of AD in which synapse damage leads to clinical symptoms in the absence of any gross loss of neurons. The concentration of $A\beta$ in these experiments was critical for two reasons. Firstly, the trafficking of $A\beta$ may be concentration dependent, as supra-physiological concentrations of AB may induce abnormal trafficking pathways. Secondly, it is important to understand the trafficking of $A\beta$ in normal rather than dying neurons

In control neuronal cultures, synaptic density was reduced after a few hours and remained suppressed throughout the 5 days of the experiment. In contrast, when neuronal cultures treated with A β oligomers for 24 hours (resulting in the loss of synapses) were subsequently treated with 200 nM squalestatin, a cholesterol synthesis inhibitor, synapse density was significantly higher after 5 days (**Figure 1**). These results demonstrate a close correlation between the removal of A β from neurons and synapse regeneration.

The gradual accumulation of $A\beta$ within the brain leads to synapse degeneration and the progressive dementia associated with AD. The observation that $A\beta$ oligomers are stable in neuronal lipid rafts and traffic *via* a recycling pathway that avoids the lysosomes may be an important factor in AD pathogenesis as it suggests that $A\beta$ is cleared slowly from neurons. The demonstration that pharmacological manipulation of neurons can dramatically increase the rate of $A\beta$ degradation and subsequently facilitate the regeneration of synapses may have important therapeutic

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Figure 1 Synapse density is restored in treated neurons.

The amounts of synaptophysin (indicative of synapse density) in mouse cortical neurons treated with control medium (\Box) or with 200 nM squalestatin, a cholesterol synthesis inhibitor () 24 hours after the addition of 10 nM A β . Values are the mean \pm SD from triplicate experiments (n = 6). *Synaptophysin significantly higher than in control neurons.

implications. Although many experimental approaches block the development of pathology, in the real world therapeutic intervention will only be effective if it can reverse existing pathological changes. Reducing intraneuronal concentrations of AB may provide a mechanism that allows synapse regeneration.

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